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The Host Range of *Phomopsis cirsii*; A Potential Biological Control Agent of *Cirsium Arvense*

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Abstract *Cirsiumarvense* is a noxious perennial weed which has become an increasing problem in North European countries partly because of restriction in use of effective herbicides.Mechanical weedingislabour intensive and expensive and therefore there is a need for an additional method likebiological control. An isolate PKDK101 of the fungus *Phomopsiscirsii,* which is virulent to *C. arvense* causing stem canker and die back was chosen to test the specificity of the fungus. A series of infection trials were successively carried out on 127 plant species (incl. ssp. and var.) belonging to 16 families in greenhouses in order to encircle the host range of *P. cirsii*. Susceptible plant species were found only in the thistle group (*Cardueae*) which contained 34 species belonging to 12 genera. Susceptible species were found in thirteen of these genera. Highly susceptible species included *Carduusacanthoides, Carduuspycnocephalus, Cirsiumeriophorum, Cnicusbenedictus, Galactitestomentosa, Notobasissyriaca, Silybummarianum* and *Tyrimnusleucographus,* which showed symptoms from girdling of stem, heart rot in rosettes to death of entire plants. Mild and restricted symptoms were observed on *Carduuscrispus, Carduusnutans, Cirsium echinus, Cirsiumvulgare* and *Cynaracardunculus*var.*scolymus* (artichoke) with symptoms such as restricted necrotic leaf spots and too early senescence or death of entire leaf. Eleven hosts for *P. cirsii* were recorded but despite the expanded range of hosts we expect that its host range will be within *Cardueae.P.cirsii,*poses multi-target potential against several annual and biennial weedy thistles from warmer climates. The pathogenicity of *P. cirsii* towards the artichoke, however, could limit its field of application especially in the Mediterranean area. The potential of *P. cirsii* as a control agent, in areas where artichokes are cultivated, would depend on the existence of *P.cirsii* resistant varieties or the existence of *P.cirsii*isolates non-pathogenic to artichoke.

Keywords BiologicalControl, Canada Thistle, *Phomopsiscirsii*, Host Range, Multi-Target Potential, Mycoherbicide

1. Introduction

Cirsiumarvense (L.)Scop.is one of the world's most troublesome and persistent perennial weeds[1],[2]. In dense stands crop loss can exceed 70%[3]. Contamination of seed, grain or crop straw reduces quality, and spines are a source of physical damage to animals. *C. arvense* has become an increasing problem in North European countries especially in organic agriculture[4],[5],[6],[7],[8]. The plant produces an extensive far-creeping and deep root system, which insures survival and rapid vegetative spread. New aerial shoots can arise at any point along the horizontal root resulting in dense patchesonly a few years after infestation [2],[9].

Long distance dispersal of the plant happens from pieces of roots as well as seeds [10].Restrictions in use of effective herbicides (*e.g.*phenoxy-herbicides), the increasing area with organicagriculture and the widespread establishment of set-asideduring the 90'ties and the beginning of this century are possibly responsible for the increasing abundance of *C. arvense* on arable land in the Nordic countries[4],[5], [11],[12],[13].

In organic cropping systems repeated cultivation or cutting are used to starve the roots and prevent further shoot emergence and assimilation [11],[14]. Such treatments are labour intensive, expensive, and require the right equipment which many farmers do not have. Hence, there seems to be need for alternative or additional control methods in arable cropping systems.

Several pathogens with potential as biological control agents have been studied such as *Sclerotiniasclerotiorum* (Lib.) de Bary(*e.g.*[15],[16]), *Alternariacirsinoxia* Simmons & Mortensen[18],[19], *Pucciniapunctiformis* (Str.) Röhl. (*e.g.*[20]) and *Phomadestructiva*Plowr.[21], but none of these pathogens have been developed into effective mycoherbicides against *C. arvense.*

Phomopsiscirsii Grove is commonly found on diseased *C. arvense* in Denmark[22] and representative isolates of this fungus have been verified by Dr. E. Punithalingam at the International Mycological Institute (IMI), and isolates were deposited (IMI no. 287751 and 278416) for patent purpose

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[23]. Findings have also been recorded on this host from Norway[24] and England[25]. Adding to these findings, *P.cirsii* has been recorded from *Cirsiumpalustre* L. (Scop.) in Norway[26], *Cirsiumeriophorum* L. (Scop.) in England[24] and more recently on *Cirsiumvulgare* (Savi.) Ten.in Germany[27]. Its virulence and aggressiveness towards *C. arvense* has been proven in glasshouse trials. An isolate (PKDK101) were able to kill all infested *C. arvense* plants within 21 days. The first symptoms appeared 5–7 days after inoculation, typically as dark brown or black spots or stripes on the leaf veins, most frequently on the young leaves or the stem. The fungus invaded the stems directlyor most frequently via the leaf veins and after girdling of the stem, it always grew downwards towards the roots, causing gradual die back of the shoots[22].

Approximately 65 of the species of *Phomopsis* listed by Uecker[28] are considered to be plant pathogenic and host specific. So far, at least four species have been investigated for potential as bioherbicide agents. Shivas et al.[29] demonstrated that *P. emecis*Shivas, the causal orgasms of stem blight of the noxious weed *Emexaustralis*Stein., was pathogenic to five closely related species in the *Polygonaceae,* and that inoculation of other unrelated plant species resulted in infection only when the plants were wounded or were senescent, and that the organism did not advance to the healthy tissues.

The fungus *Phomopsisamaranthicola* Rosskopf, Charuda ttan, Shabana, & Benny targeting *Amaranthus* spp. has been patented for *Amaranthus* control[30, 31]. Host range testing has been performed on 21 species in the genus *Amaranthus* and 56 plant species outside the genus *Amaranthus,* including crops, and members of genera that are closely related to *Amaranthus*. *P.amaranthicola* did not infect any of the plants from outside the genus *Amaranthus* but was highly pathogenic to several of the species in the genus *Amaranthus*[32]. The pathogen has shown varying efficacy. Despite plants being given an initial dew period, *P. amaranthicola* did not cause mortality on any *Amaranthus* species in greenhouse or under field conditions in experiments conducted in south Texas [33].

A large number of isolates of *Phomopsis*sp. has been collected from the weed*Carthamuslanatus* L. (saffron thistle) in Australia, and their potential as biological control agents against weeds of the *Asteraceae* has been demonstrated[34].

The susceptibility of *Convolvulus arvensis* L. accessions from different geographic locations to disease caused by the fungal pathogen, *Phomopsis convolvulus*Ormeno, has been evaluated[35]. The emerging shoots of accessions showed severe disease development and the fungal application on Greek and Montana accessions reduced aboveground biomass 83 to 100% and 65 to 86%, respectively. Results of this study indicate that control of *C. arvensis* using *P. convolvulus* might be achieved in various geographic regions [35]. Conclusively, *Phomopsis* spp. may be candidates as bioherbicides for several weed species.

The objective of this study was to determine the host range of *P.cirsii*since biological control agents should be environmentally safe and unwanted side-effects on the wild flora, crops and ornamental plants should be avoided.

2. Material and Methods

A series of experiments were carried out in order to define the host range of the fungus.The host range was evaluated qualitatively in greenhouses on a selection of available crop, ornamentals and wild plant species tested according to the centrifugal phyllogenetic scheme suggested by Whapshere [36].

2.1. Plant Material

A range of test plants belonging to 108 species and 37 genera were propagated from seeds, tubers or roots. Seeds of plants exotic to Denmark were either provided by the Botanic Garden, University of Copenhagen or bought at seed stores. Seeds of endemic wild plants were collected locally and crop plants grown in Denmark were provided by the Faculty of Sciences, University of Copenhagen or from local seed stores.

Healthy looking seeds from the dicotyledonous plants were sown in trays in a 1:3 mixture of gravel and peat soil (Pindstrup no. 2, pH 5.6-6.6) and healthy looking seedlings at the two true leaf stage were transplanted into 13 cm diameter plastic pots. Plants of *C. arvense* were cloned from 3-5 cm long root pieces with at least two root buds. Monocot plants were established in 13 cm diameter pots containing 10 seed per pot and grown without transplanting. The plants were grown under greenhouse conditions with supplemental lighting 12 hours day^{-1}, supplied by 400 Watt Phillips mercury lamps. Day and night temperatures fluctuated between 13 and 33℃ with means of 16-20℃ and 20-25℃ respectively. Pests were controlled using yellow sticky traps. The plants were watered individually according to requirement.

2.2. Inoculum Production

The fungus *P.cirsii* isolate PKDK101[22] was used in all 10 trials. The fungus was cultivated in Roux glass bottles on the surface of 250 ml of sterile CzapeckDox Broth with 0.01 % DifoBacto agar (DifcoMicrobiology). The bottles were inoculated with four plugs (4 cm^2) cut from actively growing margins of colonies (fig. 1B) on Potato Dextrose Agar(PDA) and incubated for 4-5 weeks at 20-25°C in diffuse light in the laboratory. The resulting mycelial mats were then harvested and prepared for inoculation as described by Lethet al.[22]. The final inoculum consisting of a suspension of mycelial fragments was adjusted with sterile deionised water to contain 80 g of mycelium per litre (0.08 g ml^{-1}).

2.3. Inoculation of Plants

The virulence and aggressiveness of *P.cirsii* isolate PKDK101 on *Cirsiumacaule*(L.) Scop.,*Cirsiumcarlinoides* Fisch. and *Carduusthoermeri* Weinm. wastested using three

to six plants due to unavailability of sufficient numbers of seeds. All other tests were done using 10-15 test plants per species and variety. For cereals five pots were sprayed. As a control, the same numbers of the plants in question were sprayed to run off with deionised water. In order to confirm the pathogenicity of the inoculum, five plants of *C. arvense* grown from root pieces of a susceptible Danish clone were co-inoculated at each of the ten successive infection trials. The infection trials were carried out one to two weeks apart and for annual plants at the six leaves to flower bud stage; for biennial and perennial plants in the rosette stage. Cereals were tested when they had developed four to six leaves. The plants wereinoculated by spraying to run off with the mycelia suspension, using compressed air (2 kg cm^{-2}) and a spray gun.The inoculated plants were then covered by polyethylene bags and incubated 72 hours under greenhouse conditions before removal of the bags. During the daytime the plants were protected against sunlight using sheets of white paper while incubated in the plastic bags. As quality assurance, an experiment was accepted when at least three out of the five *C.arvense* control plants showed symptoms of infection 14 days after inoculation (DAI), otherwise the same plants were re-inoculated with a new batch of inoculum.

2.4. Disease Rating

The inoculated plants were evaluated for disease symptoms 21 DAI according to the previously developed disease severity scale for the *P.cirsii- C.arvense*pathosystem (Table 1)[22].

Table 1. Disease severity rating developed to quantify infection of *Cirsium arvense*by *Phomopsiscirsii* (from[22])

Rating	Symptoms
0	No symptoms
1	Restrictedleaf spots/necrosis
2	Leaf spots with some necrosis (blackening) of secondaryleafveins
3	Secondary leaf veins necrotised as far as the mid-leafvein
4	Secondary leaf vein and some mid leaf vein necrotised
5	Mid vein necrotised as far as the leaf base
6	Death of entireleaf
7	Invasion of stem directly or via leaf vein
8	Longitudinal necrotisation of stem cortex
9	Girdling of stem, heart rot in rosette
10	Necrotisation from girdling point towards base of shoot
11	Death of shoot for both perennial and annual plants
12	Die back of re-grown shoots
13	Death of entire plant

Re-isolation was carried out from plants with visible symptoms. Infected plant parts were surface sterilised with 70 % ethanol for 30 sec. and transferred to 2 % NaOCl for one minute, cut into small pieces of a few millimetres length and plated on PDA in Petri dishes. The Petri dishes were placed in the laboratory at 20-25°C in diffuse light and observed under stereo microscope at intervals over the following five days for the presence of typical *P. cirsii* mycelia emerging from the plant tissue and later for pycnidia with alfa and beta-conidia[22].

Symptomless plant species and their set of control plants were kept for observation until senescence occurred. Plants of biennial and perennial thistle species in the rosette stage without symptoms were kept for observation in the green house for an additional year.

3. Results

Table 2. Disease severity (See Table 1) of greenhouse plants of species belonging to Tubuliflorae part of the tribe Cardueae inoculated with *Phomopsiscirsii* isolate PKDK101. Evaluation was done 21 days after inoculation. Names follow[39] and[40]

Cardueae	Rating
Carduusacanthoides L.	1-13
Carduuscrispus L.	2-3
Carduuscrispus L. *f. alba*	2-3
Carduusdefloratus L. ssp.*carduelis* (L.) Kelm.	0
Carduuspycnocephalus L.	9-13
Carduussquarosus (Ds.) Lowe.	0
Carduusnutans L. subsp. *leiophyllus* *	5
Carthamuslanatus L.	0
Carthamustinctonus L.	0
Centaureacyanus L.	0
Centaureaodorata Burm.f..	0
Centaurea sp.	0
Cirsiumacaule Scop.	0
Cirsiumarvense(L.) Scop.	10-12
Cirsiumechinus (Bieb.) Hand-Mazz.	2-3
Cirsiumdiscolor (Muehl) Spreng.	0
Cirsiumeriophorum (L.) Scop.	6-13
Cirsiumhelenoides (L.) Hill.	0
Cirsiumoleraceum (L.) Scop.	0
Cirsiumpalustre(L.) Scop.	0
Cirsiumvulgare (Savi.) Ten.	2-3
Cnicusbenedictus L.	9-13
*Cynaracarduncul*usL.	0
*Cynaracarduncul*usL. var*scolymus* (cv. Amelione)	0
*Cynaracarduncul*usL. var*scolymus*(cv. Green Globe)	2-3
Echinopsritro L.	0
Galactitestomentosa (L.) Moensch.	9-13
Notobasissyriaca (L.) Cass.	9-13
*Onopordonacanthium*L.	0
*Onopordonalgeriense*Pomel	0
Onopordonillyricum L.	0
*Onopordontauricum*Willd.	0
Silybummarianum (L.) Gaertn.	9-13
Tyrimnusleucographus (L.) Cassini	9-13

* only three plants available

The type of symptoms on the susceptible plants was identical to the reaction types seen in previous studies on *C. arvense* (Table 1). Thirteen of the thistle species (*Cardueae*) tested (Table 2), were susceptible to *P. cirsii* showing various degrees of disease severity reactions except for *Carduuscrispus* L. and its white flowered variety (*f. alba*) which expressed distinct yellow halos around the patches of infected, necrotic leaf veins. *P. cirsii* was successfully re-isolated from all symptoms, confirming the virulence towards the tested plants. The disease severity for *C. arvense* control plants was not rated higher than 12 due to re-growth of aerial shoots from the roots in these experiments. As

expected *Cirsiumeriophorum*, the previous described host for *P. cirsii*[25], was highly susceptible to the fungus, contradictory to the results obtained for *Cirsiumpalustre* (L.) Scop., which was resistant to the fungus despite previous findings of *P. cirsii* on this host [26].

Of the thirteen new hosts for *P. cirsii* recorded, *Carduusa canthoides* L., *C. pycnocephalus* L., *Cnicusbenedictus L., Galactitestomentosa* (L.) Moensch., *Notobasissyriaca* (L.) Cass., *Silybummarianum* (L.) Gaertn. and *Tyrimnusleucogr aphus* (L.) Casso were highly susceptible and to the same degree as the *C. arvense* control clone. Six species were categorized having low susceptibility expressing restricted necroses on leaf veins or/and restricted leaf spots. These were: *Carduuscrispus* and its white flowered variety, *Cirsiumthoermeri*, *C. carlinoides*, *C. echinus* (Bieb.) Hand-Mazz., *C. vulgare* and*Cynaracardunculus*var*scolymu s* L. Of the latter species the cultivar Green Globe was attacked while the cultivar Amelione appeared resistant. The rest of the *Cardueae*testplants listed in table 2, as well as all test plants outside *Cardueae*(Table 3) were resistant.

4. Discussion

No matter how effective the biological control agent is, host specificity remains the crucial filter for the selection of biological control agents. Unwanted side-effects on non-target plant species may have serious consequences for the food-web in the ecosystem and economic consequences for the society. As a consequence of increasing awareness of possible side-effects of biological control, the degree of acceptable risk, tolerated by regulatoryauthorities is becoming less and less, even in countries where biological weed control has been widely accepted and successful (*e.g.*[37],[38]). According to Whapshere[36] it should be expected that a bio-control agent isrelativelyspecific ifit only attacks some of the plant species closely related to the target plant.However, due to the weak species concept of the form-genus *Phomopsis*and its importance as pathogens ofmany different crop plants[22],[28], it was decided that the present experiments should include an extended number of plant genera and species.

Whapshere´s assumption did hold true for the present *P. cirsii* isolate which kept its host range within the tribe *Cardueae,* and even with great variation in susceptibility of the closest related species to *C. arvense* (*Cirsium*, *Carduus*), reaching from resistant (0) to highly susceptible (9-13) Increasing knowledge about the biology of the genus *Phomopsis* has revealed that some species are endophytes and invaded the host without creating symptoms or resulting in latent infections on some ofthe apparently resistantspecies of the *Cardueae,* especially,on the true thistles*Cirsium* and *Carduus* spp..However, none of the symptomless plants expressedsymptoms during the prolonged incubation period, until senescence occurred. The host preference may vary among isolates of *P. cirsii,* but can be expected to remain within *Cardueae*. Previous studies have shown that the cultivation conditions of the fungus may influence its virulence [22]. In the present series of inoculations of test plants the PKDK101 isolate remained virulent to the *C. arvense* control plants, except for one set of plants, which had to be successfully re-inoculated.

Table 3. Greenhouse plants of species which did not show any symptoms after inoculated with Phomopsiscirsii isolate PKDK101. Evaluation was done 21 days after inoculation

Groupofplants	Speciestested	Varietiestested
ASTERACEAE		
Tubuliflorae		
Cardueae	Seetable2	
Heliantheae	*Galinsogaparviflora*Cav.	
	Galinsogaciliata(Raf.)Blake	
	*Heliathusannuus*L.	cv.Bismarckianus
	*Helianthustuberosus*L.	cv.Unknown
	*Zinniaelegans*Jacq.Fl.Pl.	cv.Kaliforniskkæmpe,cv.Persiancarpet
Anthemideae	*Achilleamillefolium*L.	
	*Anthemisarvensis*L.	
	*Artemisiavulgaris*L.	
	*Chrysanthemumcarinatum*Schousb.	cv.Regnbue
	*Chrysanthemumleucanthemum*L.	
	Chrysanthemumpaludosum	
	*Chrysanthemumsegetum*L.	
	*Matricariachamomilla*L.	
	Tripleurospermumperforatum(Mérat)Lainz	
Astereae	*Calistephuschinensis*Nees.	cvs.Burpeana,Remo,Strudsfjer,Unikum,Prinesse.

Calenduleae	*Calendulaofficinalis*L.	cv.unknown
Helenieae	*Tagetespatula*L.var.Nana	cv.Fiesta
Inuleae	*Acroclinium roseum* Hook.	cv.unknown
	*Rhodanthemanglesii*Lindl.	cv.unknown
Senecioneae	*Cinerariamaritima*L.	
	*Doronicumpardalianches*L.	cv.unknown
	*Senecio jacobea*L.	
	*Senecio vernalis*L.	
	*Senecio vulgaris*L.	
	*Tussilagofarfara*L.	
Liguliflorae		
Cichorieae	*Cicerbinaalpina*(L.)Wallr.	
	*Cichoriumendivia*L.	cv.CurledMeaux
	*Cichoriumintybus*L.	
	Lactuca sativa L. var. capitata	cvs.HjerterEs,Pennlake
	*Lapsanacommunis*L.	
	*Scorzonerahispanica*L.	cv.Russiskkæmpe
	*Sonchusarvensis*L.	
	Sonchusasper(L.)Hill.	
Table3continued Groupofplants	Speciestested	Varietiestested
	*Sonchusoleraceus*L.	
	*Taraxacumofficinale*Weber	
	*Tragopogonporrifolius*L.	
	*Tragopogonporrifolius*L.ssp.Australis	
CAMPANULACEAE	*Campanularapunculoides*L.	
CANABACEAE	*Canabissativa*L.	cv.unknown
CHENOPODIACEAE	*Betavulgaris*L.	cvs.MariboPoly,Alfashort,KyrosPajbjergyellow
	*Betavulgaris*L.var.esculenta	cvs.Cylinder,Unik,Toftø

Table 3 continued

Groupofplants	Speciestested	Varietiestested
	*Spinaciaoleracea*L.	cvs.Freja,ØtofteS77
CONVOLVULACEAE	*Convolvulustricolor*L.	
CRUCIFERAE	*Brassicajuncea*(L.)Czern.	
	*BrassicanapusL.xB.napus*L.ssp.*oleifera*(Metzg.) Sinsk.	
	*Brassicanapus*L. ssp.*oleifera*(Metzg.) Sinsk.	cvs. Quinta, SvaløfDuro, Karat, SvaløfBeke
	Brassicanapus L. var. *napobrassica* (L.) Peterm.	cvs.BangsholmRutaØtofteS70,WilhelmsbergSatorØtofte
	Brassicanigra(L.)Koch	
	Brassicaoleracea L.convar.*acephala D.C.*	cv.GrünerAngeliter
	*Brassicaoleracea*L.var.*gemmiferazenker*	cv.HuginToftø
	Brassicaoleracea L.var. *capitata f. alba* L.	cvs.WinterLangendijker,ErstlingErnora
	*Brassicaoleracea*L. var. *Capitata f. rubra* L.	cv.HoldbarAmager
	*Brassicarapa*L. var.*rapa*(L.) Thell.	cvs.FynskBortfelderRanaDaehnfeldt,ØsterSundomKava Daehnfeldt
	Capsellabursa-pastoris(L.)Med.	
	Camelinasativa(L.)Ortz.	cv.SvaløfCamé
	*Crambehispanica*L.	cv.Unknown

	*Raphanussativus*L.	cv.RundKøbenhavnTorve
	Raphanussativus L. ssp.*Oleifera* (D.C.) Metzg.	cv.Siletina
	*Sinapsisalba*L.	cv.Alba
CUCURBITACEAE	*Cucumissativus*L.	cv.LangelandsKæmpeToftøS75
DIPSACACEAE	*Scabiosaatropurpurea*L.	cv.unknown
	*Scabiosastellata*L.	cv.unknown
EUPHORBIACEAE	*Euphorbiahelioscorpia*L.	
GRAMINEAE	*Avenasativa*L.	cv.HedwigWeibull
	*Hordeumvulgare*L.	cvs.Bonus,Pallas,CajaPajbjerg
	*Secalecereale*L.	cv.unknown
	Setariaitalica(L.)Beauv.	cv.unknown
	Sorghumbicolor(L.)Moench.	cv.Hybrid31
	*Triticumaestivum*L.	cv.unknown
Table 3 continued Groupofplants	Speciestested	Varietiestested
	*Zeamays*L.	cv.LG11
LILIACEAE	*AlliumascalonicumL.*	cv.unknown
LINACEAE	*Linumusitatissimum*L.	cvs.Wiera5215,andanunknowncultivar
PAPILIONACEAE(LEGUMINOSAE)	*Ervumlens*L.	cv.Unknown
	Glycinehispida(Moench.)Maxim.	cv.FiskebyV.
	*Lotuscorniculatus*L.	cv.TidligØtofte
	*Lupinusluteus*L.	cv.Weiko
	*Medicagolupulina*L.	cv.VirgoPajbjerg
	*Medicagosativa*L.	cv.Vela
	*Melilotusalba*Med.	cv.unknown
	*Onobrychissativa*Lam.	cv.unknown
	*Ornithopussativus*Brot.	cv.unknown
	*Phaseolusvulgaris*L.	cv.FrucaSimploS69,Bef
	*Pisumsativum*L.	cv.MultiStarDaehnfeldt

Table 3 continued

Groupofplants	Speciestested	Varietiestested
	*Trifoliumpratense*L.	cvs.KranoPajbjerg,TiloDaehnfeldt
	*Trifoliumrepens*L.	cv.MilkanovaPajbjergK&VS70
	*Trifoliumhybridum*L.	cv.ErmoØtofteS69
	*Viciafaba*L.	cv.Ackerperle
	*Viciasativa*L.	cv.unknown
POLYGONACEAE	*Fagopyrumesculentum*Moench.	cv.unknown
SOLANACEAE	*Nicotianarustica*L.	cv.unknown
	*Nicotianatobacum*L.	cv.unknown
	*Solanumtuberosum*L.	cv.Bintje
UMBELLIFERAE	*Carumcarvi*L.	cv.unknown
	Daucus carota L. var. *Sativa* DC	cv.FeoniaDanaToftøS74

In the present study re-isolation took place only from species which showed symptoms of infection. However, we suggest that in future studies it should be taken into consideration that the fungus may appear latentlyin symptomless plant tissue and thus potentially result in a physiological effect such as biomass reduction on its host. No traces of infection or visible negative effects were noted on the resistant plants within the *Cardueae*or on any other test plants belonging to the 16 plant families outside *Cardueae*. The critical species in *Cardueae* are *Carthamusti*

nctorius (safflower) grown in warm and dry areas of the world and used for ornamental purposes, colour pigments and precious edible seed oil and *Cynaracarduncul*us L. (cardoon); a valued vegetable from which extracts are used for coagulants (enzymes) in cheese production[41], and more recently a candidate for production of bio-fuel[42]. Despite their importance both plant species are also listed as noxious weeds[43]. Both species were resistant towards the PKDK101 isolate. However, the closely related *Cynaracard unculus*var.*scolymus* (artichoke), an important vegetable crop of the Mediterranean area and California[44],[45],[46], expressed symptoms of infection, though to a minor degree, with restricted blackening of secondary leaf veins and a few black stripes on the leaf mid veins, symptoms which caused early senescence of the infected leaves, but which did not progress during the prolonged one-year observation period.

Highly susceptible plants (grade 8 to 13) (Table 1) are all annual weedy species registered on the list of world weeds[1] except for the annual *Tyrimnusleucographus* (L.) Cassini, which grows in sandy and stony habitats in the Mediterranean area[40].

The results indicate that a mycoherbicide based on *P. cirsii* may have potential as control agent againstseveral weedy species of thistles.

Unlike several other *Phomopsis* species that have been reported as pathogens of plants from more than one plant genus[25],[47],[48], *P.cirsii* exhibited a high degree of specificity only to one genus (Cardueae) (Table 2) like *P. amaranthicola,*which exhibitedhigh degree of specificity only to the genus Amaranthus[32].

A large number of isolates of *Phomopsis* sp. was collected, and analyses of their geneticdiversity showed minimal variation between them, except for two isolates that appeared to shareidentity with the teleomorph*Diaporthehelianthii* and with *P. viticola*[34]. A multigene phylogenetic analysis and comparison between the isolates from Australia and Denmark would be useful to unravel relationship.

Host-specificity testing may be adequate for determining physiological host range, but may fall short on predicting ecological host range. This is because a variety of factors can influence selection of hosts under natural conditions, including phenological synchrony, host and agent dispersal, habitat type and life history variation. Several case studies of biological control have shown that host-specificity tests in quarantine, which suggest a broad host range for a biological control agent, are not necessarily indicative of a wide field host range[49]. Study of the ecological host range of *P. cirsii* will be one of the next steps in order to unravel the potential of *P.cirsii* as a bioherbicide.

5. Conclusions

The results from this study show that *P.cirsii,* which to our knowledge hasonly been recorded from thistlesinDenmark, England, Germany and Norway[22],[25],[26],[27],poses multi-target potential against several annual and biennial weedy thistlessuch as *Cirsiumarvense*, *Carduuspycnocepha lus* L., *Cnicusbenedictus*L., *Galacitestomentosa*(L.) Moensch, *Notobasissyriaca* (L.) Cass and *Silybummarianum* (L) Gaertn..The *P. cirsii* isolate kept its host range within the tribe *Cardueae,* and with great variation in susceptibility of the closest related species to *C. arvense* (*Cirsium*, *Carduus*), reaching from resistant (0) to highly susceptible (9-13). The pathogenicity of *P. cirsii* towards the artichoke, however, could limit its field of application especially in the Mediterranean area. The potential of *P. cirsii* as a control agent, in areas where artichokes are cultivated, would depend on the existence of *P.cirsii* resistant varieties or the existence of *P.cirsii*isolates non-pathogenic to artichoke. Further studies should include repeated specificity tests with different isolates of *P. cirsii* concentrating on crop plants from the *Cardueae*group (*Cynara* spp. and *Carthamus* spp.) and on endangered thistle species.

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