Interaktionen zwischen der Ackerkratzdistel, pathogenen Pilzen und phytophagen Insekten: Grundlagen einer biologischen Unkrautkontrolle

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Einleitung

Das Interesse an einer Reduktion von Unkräutern mit Hilfe von natürlichen Gegenspielern, der biologischen Unkrautkontrolle, hat in den letzten Jahren stark zugenommen (z. B. DeBach 1974, Huffaker & Messenger 1976, Burges 1981, Burge 1988, Te Beest 1992, Murdoch 1996, McEvoy & Coombs 1999, Charudattan & Dinoor 2000, Headrick & Goeden 2001, Müller-Schärer et al. 2000). Als Unkräuter werden dabei solche Pflanzen bezeichnet, die durch ihr unerwünscht häufiges Auftreten auf landwirtschaftlichen Flächen spürbare Ernteeinbußen verursachen. Eine zunehmende Zurückhaltung gegenüber dem Einsatz von Herbiziden und die fortbestehende Unkrautproblematik aufgrund eingeschränkter Einsatzmöglichkeiten von Herbiziden in Naturausgleichsflächen und Flächen des biologischen Landbaus (Bacher et al. 1997) haben die Suche nach alternativen Kontrollmöglichkeiten für Unkräuter angeregt (Frantzen 1994a, Müller-Schärer & Frantzen 1996). Erfolge im Bereich der Klassischen Biologischen Kontrolle haben auch eine biologische Kontrolle für einheimische Unkräuter möglich erscheinen lassen. Bei der Klassischen Biologischen Kontrolle werden eingeschleppte Unkräuter, die sich durch das Fehlen natürlicher Feinde ungehemmt ausbreiten konnten (invasive Arten), durch ein Nachführen spezialisierter Gegenspieler reduziert (Watson 1991). Im Gegensatz dazu werden beim inundativen bzw. augmentativen Ansatz der biologischen Kontrolle ausschließlich einheimische Gegenspieler einer Art eingesetzt (Charudattan 1988). Unter inundativer Kontrolle versteht man ein massenhaftes Ausbringen der Antagonisten, zum Beispiel auf einjährig genutzen Flächen im Ackerbau. Durch inundative und wiederholte Applikationen können beispielsweise pathogene Pilze als sogenannte Mykoherbizide wirken (Charudattan & Dinoor 2000). Als augmentativ bezeichnet man einen Ansatz, bei dem geringere Mengen eines Gegenspielers ausgebracht werden, der durch seine natürliche Verbreitung jedoch ebenfalls zur Kontrolle einer Art beiträgt. Biologische Kontrolle zielt nicht darauf ab, Unkrautpopulationen völlig zu beseitigen, sondern versucht, sie auf ein für die Landwirtschaft verträgliches Maß zu reduzieren. Damit trägt sie dem gewandelten Verständnis einer umweltverträglichen Landwirtschaft Rechnung (Müller-Schärer & Frantzen 1996).

Bei den Gegenspielern, die im Zuge einer Biologischen Kontrolle eingesetzt werden, kann es sich um Insekten oder auch pathogene Pilze handeln. Eine Vielzahl von Untersuchungen hat sich mit einer Kontrolle durch Herbivore (z.B. Überblick für die Cardueae in Schroeder 1980, Peschken et al. 1982, Ang et al. 1995, Peschken et al. 1997) als auch durch Pathogene (z.B. Burdon et al. 1981, Frantzen 1994a, Barreto & Evans 1998, Harvey et al. 1998, Jahromi et al. 1998, Fauzi et al. 1999, Frantzen & Müller-Schärer 1999, Hurrel et al. 2001) befaßt. Dennoch gibt es erst in letzter Zeit eine vergleichsweise geringe Anzahl von Untersuchungen, die die möglichen Interaktionen zwischen Pilzen und Insekten und ihre Auswirkungen auf die gemeinsame Wirtspflanze berücksichtigen (Chang & Thrower 1981, Bultman & White 1988, de Nooi 1988, de Nooi & van Damme 1988, Ramsell & Paul 1990, Zebitz 1990, Prüter & Zebitz 1991, Hatcher et al. 1994a, Hatcher 1995, Kok et al. 1996, Paine et al. 1997, Moran 1998, Friedli and Bacher 2001a, Hatcher and Paul 2001, Kluth et al. 2001, Kruess 2002). Dabei könnten diese Wechselwirkungen, sei es zwischen natürlicherweise vorhandenen oder zwischen künstlich ausgebrachten Organismen, den Erfolg oder Mißerfolg der Biologischen Kontrolle entscheidend beeinflussen. So kann beispielsweise die erstrebte Schadwirkung durch die eingesetzten Gegenspieler verringert sein, wenn diese Organismen sich in ihrer Wirkung gegenseitig hemmen. Desweiteren könnten die Auswirkungen des Gegenspielers von der An- oder Abwesenheit anderer Arten auch unbeeinflußt sein (äquivalente Wirkung), die Schadwirkung der Gegenspieler kann additiv sein, oder aber in ihrer Gesamtwirkung noch über einen additiven Schaden hinausgehen (synergistische Wirkung, Hatcher 1995). Mutualistische oder antagonistische Beziehungen zwischen den Organismen können so den Effekt der biologischen Kontrolle positiv oder negativ beeinflussen.

Die Organismen

In dieser Arbeit sollen grundlegende Fragen einer biologischen Kontrolle der Ackerkratzdistel (*Cirsium arvense* (L.) Scop. Abb. 1) durch pathogene Pilze und phytophage Insekten untersucht werden. Die Ackerkratzdistel ist eine in den gesamten gemäßigten Breiten auftretende Art, die sich sehr erfolgreich sowohl auf sexuellem als auch auf vegetativem Wege ausbreiten kann. Sie gilt weltweit als bedeutendes Unkraut (Holm et al. 1977). Der Einfluß der sexuellen Fortpflanzung für eine Verbreitung der Ackerkratzdistel ist noch nicht abschließend geklärt und möglicherweise von geringer Bedeutung (Donald 1990, Oesau 1990), da die Flugfähigkeit der Samen durch ihren leicht abbrechenden Pappus stark eingeschränkt ist (Bakker 1960, Moore 1975). Zudem tritt die Distel, die unvollkommen zweigeschlechtlich ist (Lloyd & Mayall 1976), häufig in ausgedehnten Klonen auf, wodurch die Wahrscheinlichkeit einer

Abb. 1 Die Ackerkratzdistel *Cirsium arvense* (L.) Scop. (verändert nach Holm et al. 1977).

erfolgreichen sexuellen Befruchtung der Samen herabgesetzt ist. Die Keimlinge der Distel sind sehr empfindlich gegenüber der Konkurrenz durch andere Pflanzen, insbesondere durch Beschattung (Holm et al. 1977). Eine Etablierung von Distelkeimlingen ist daher vor allem auf offenem Boden und an gestörten Stellen wie Ruderalflächen und Segetalfluren zu erwarten (Moore 1975). Im Gegensatz dazu verbreitet sich die Ackerkratzdistel sehr erfolgreich durch ihr weitreichendes unterirdisches System von verdickten Horizontalwurzeln (Moore 1975) und ist in Acker- und Grünlandflächen, aber auch auf ökologischen Ausgleichsflächen nur schwer zu kontrollieren. In den Horizontalwurzeln werden im Jahresverlauf Speicherstoffe eingelagert (Otzen & Koridon 1970, McAllister & Haderlie 1985). Bereits aus Fragmenten einer Länge von wenigen Zentimetern, wie sie z.B. durch eine Zerteilung der Horizontalwurzeln im Zuge der landwirtschaftlichen Bodenbearbeitung entstehen, können sich daher ganze Pflanzen regenerieren (Hamdoun 1972, Kluth et al. 2002a, b). Eine vollständige Bekämpfung der Distel ist bislang nur durch den wiederholten Einsatz von Herbiziden zu erlangen (Donald 1994).

Der Rostpilz *Puccinia punctiformis* (Str.) Röhl (Uredinales) ist ein weitverbreiteter und für die Ackerkratzdistel spezifischer pathogener Pilz (Buller, 1950). Durch seine Spezifizität stellt er einen idealen Kontrollorganismus für die Distel dar. *P. punctiformis* ist im Gegensatz zu den meisten Wirtspilzen autözisch, d.h. in seinem Lebenszyklus findet kein Wirtswechsel statt (Abb. 2) und als obligat biotropher Pilz auf lebendiges Pflanzengewebe angewiesen. Hervorgerufen wird der systemische *P. punctiformis*-Befall der Ackerkratzdistel durch eine Infektion von Teleutosporen des Pilzes an den Wurzelknospen der Horizontalwurzeln (Van den Ende et al. 1987, Frantzen 1994a). Die austreibenden Sprosse sind bereits vom Pilzmyzel durchzogen. Systemisch infizierte Pflanzen weisen einen stark veränderten Habitus mit langgestreckten Internodien und einer chlorotischen Färbung der verschmälerten Blätter auf. Befallene Pflanzen sterben bereits im Frühsommer ab und gelangen äußerst selten zur Blüte (Buller 1950, Bailiss & Wilson 1967, Van den Ende et al. 1987, Kluth et al. 2001). Sporen-

lager entwickeln sich vorwiegend auf den Blattunterseiten systemisch infizierter Disteln, die zunächst Uredosporen, später im Jahr auch Teleutosporen freilassen. Durch Uredosporen wird eine lokale Infektion der Distel hervorgerufen, die sich in lokalen Läsionen der Blätter äußert und die Fitness der Pflanzen vergleichsweise wenig beeinflußt (Buller 1950). In den so entstehenden Sporenlagern werden ebenfalls sowohl Uredo- als auch Teleutosporen produziert. Eine nachhaltige Schädigung der Ackerkratzdistel wird vor allem bei einer systemischen Infektion erreicht. Durch den unterirdischen Infektionsmechanismus ist es aber bisher nur unter Laborbedingungen gelungen, Wurzelknospen systemisch durch direkte Sporenapplikation zu infizieren (Van den Ende et al. 1987, French & Lightfield 1990). Friedli & Bacher (2001a,b) berichten von einer systemischen Infektion von Trieben der Ackerkratzdistel nach Eiablage durch die Weibchen des Rüsselkäfers *C. rubiginosa* und vermuten einen Transport von Sporen des Rostes durch den Käfer.

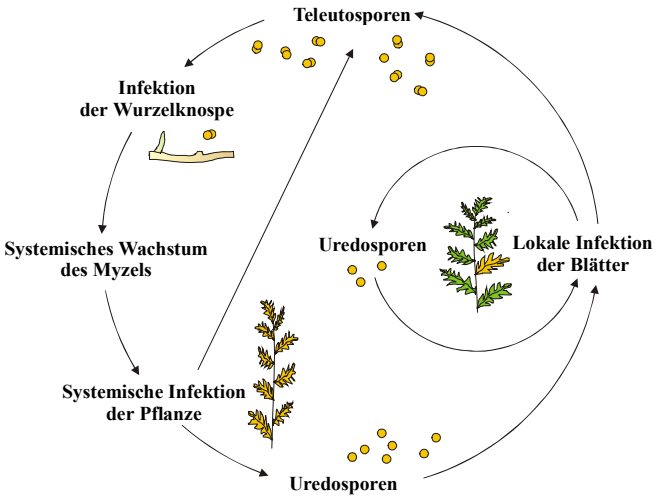

Abb. 2: Infektionszyklus des Rostpilzes *Puccinia punctiformis* (Str.) Röhl. nach Gäumann (1959, verändert) unter Auslassung der Basidien und Pyknidienstadien

Boyle & Schulz (1994) regten in ihrer Untersuchung zu alternativen biologischen Kontrollstrategien der Ackerkratzdistel an, durch eine Infektion mit *P. punctiformis* und der Sekundärinfektion mit einem weiteren pathogenen Pilz den Schaden für die Distel zu erhöhen. Ähnliche Untersuchungen, die mehrere pathogene Pilze zum Einsatz brachten, dienten dabei als Vorbild (Crawley et al. 1985, Hallett et al. 1990a,b, Hallet & Ayres 1992). Im Zusammenhang mit der Ackerkratzdistel wurde der perthotrophe, d.h. auf totem Gewebe lebende, Pilz *Phoma destructiva* Plowr. (Sphaeropsidales) untersucht (Guske 1995, Guske et al. 1996, Kruess 2002a, b). Konidiosporen von *P. destructiva* rufen eine systemische Infektion der Ackerkratzdistel mit deutlich sichtbaren Chlorosen hervor, die sich negativ auf Blüte und Fruchtansatz auswirken kann (Kruess, unveröffentlicht).

Fragestellungen

In der vorliegenden Arbeit sollen Grundlagen einer biologischen Kontrolle der Ackerkratzdistel mittels pathogener Pilze und phytophager Insekten untersucht werden. Im Einzelnen wurden folgenden Fragestellungen bearbeitet:

- Welchen Einfluß haben das Alter und der Phänotyp der Ackerkratzdistel auf die Resistenz gegenüber einem Rostpilz? Gibt es Unterschiede zwischen Topfversuchen und Freilandversuchen auf einer weiteren räumlichen Skala? (Kapitel 2)
- Welchen Einfluß hat die Kombination einer Applikation des Rostes *P. punctiformis* zusätzlich zu einer mechanischen Schädigung der Ackerkratzdistel durch Mahd auf ihre Fitness-Eigenschaften und das Maß der Reservestoff-Einlagerungen? Ändern sich die beobachteten Auswirkungen bei einer Behandlungen über zwei Jahre? (Kapitel 3)
- Welche Auswirkungen hat die gleichzeitige Behandlung der Ackerkratzdistel mit zwei Pathogenen, dem Rost *Puccinia punctiformis* und dem perthotrophen Pilz *Phoma destructiva*, in einfacher, mehrfacher und kombinierter Anwendung, auf einen Bestand der Distel unter Freilandbedingungen? Kann die Distel bei jährlich wiederholter Applikation über einen Zeitraum von 3 Jahren in ihrer Konkurrenzfähigkeit geschwächt werden? Kann durch eine Erhöhung des Grades der lokalen Infektionen der Distel mit *P. punctiformis* indirekt das Vorkommen der stark schädigenden systemischen Infektion vermehrt werden? (Kapitel 4)
- Bestehen mutualistische oder antagonistische Wechselwirkungen zwischen typischen Herbivoren der Ackerkratzdistel und dem Rostpilz *P. punctiformis*? Wie beeinflußt eine Besiedlung mit herbivoren Insekten die Anfälligkeit der Distel gegenüber dem Pilz? Können herbivore Insekten zur Verbreitung des Pilzes beitragen? Wie wirkt sich eine Pilzinfektion der Wirtspflanze auf die Entwicklung der Herbivoren aus? (Kapitel 5)

Ergebnisse

Eine erfolgreiche Inokulation einer Pflanze mit einem phytopathogenen Pilz erfordert die Anfälligkeit der Pflanze gegenüber dem Pathogen, die Virulenz des Pathogens gegenüber der Pflanze sowie geeignete Umweltbedingungen, die ein Zusammentreffen beider Organismen begünstigen (Charudattan & Dinoor 2000, Börner 1997). Grundlegende Informationen über Resistenzeigenschaften einer Pflanze sind daher die Voraussetzung für die Entwicklung einer erfolgreichen biologischen Kontrolle eines Unkrautes durch pathogene Pilze. In Kapitel 2 wird der Einfluß des Alters und des Phänotyps der Ackerkratzdistel auf ihre Anfälligkeit (Prädisposition) gegenüber dem Rostpilz *P. punctiformis* unter Freilandbedingungen auf verschiedene räumlichen Skalen untersucht: sowohl in Topfversuchen als auch auf verschiedenen Brachflächen und Ackerrandstreifen auf weiterer räumlicher Skala. Das Alter der Disteln stellte sich dabei sowohl für aus Keimlingen angezogene als auch für klonal gewachsene

Disteln als ein entscheidender Faktor für die Anfälligkeit der Ackerkratzdistel gegenüber dem Rost heraus. Junge Pflanzen waren sowohl im Topfversuch wie auch im natürlichen Bestand nahezu resistent gegenüber der Inokulation mit *P. punctiformis*, während die Resistenz mit dem Alter deutlich nachließ und sich hohe Sporenlagerdichten entwickelten. Die Anfälligkeit verschiedener Distel-Phänotypen variierte sowohl in Topfexperiment als auch im natürlichen Bestand, wobei die Anfälligkeit der getopften Disteln jedoch kaum Aussagen über die Anfälligkeit des natürlichen Bestandes auf einer regionalen räumlichen Skala ermöglichte. Dies deutet auf den großen Einfluß von Umweltparametern bei der Infektion von Pflanzen durch Pathogene hin.

Im dritten Kapitel wird der Einfluß einer Behandlung mit dem Pathogen *P. punctiformis* über einen Zeitraum von zwei Jahren, kombiniert mit einer mechanischen Behandlung, auf die Entwicklung und Speicherstoff-Einlagerung der Ackerkratzdistel untersucht. Versuche, der Distel auf mechanischem Wege, z.B. durch Mahd, beizukommen, sind in zahlreichen Studien unternommen worden (Überblick in Donald 1990). In der vorliegenden Untersuchung sollte zusätzlich zu einer einmal jährlichen Mahd der Ackerkratzdistel eine Infektion mit dem Rostpilz *P. punctiformis* induziert werden, um durch die doppelte Behandlung die Schwächung der Disteln zu intensivieren. Durch Inokulation wurde zunächst die oberirdische Biomasse der Distel reduziert, während alleinige Mahd über eine gesteigerte Wachstumsrate zu einer erhöhten Biomasse-Produktion führte. Erst eine weitere Mahd im Folgejahr reduzierte sowohl die ober- wie auch die unterirdische Biomasse signifikant. Die Blütenkopf-Ausbildung war durch einfache Mahd stark verringert, der Anteil fertiler Blütenköpfe jedoch erhöht. Hier reduzierte eine kombinierte Behandlung mit *P. punctiformis* und Mahd den Anteil fertiler Blütenköpfe signifikant. Auf die Speicherstoffe in den Horizontalwurzeln der Distel wirkte sich insbesondere die Mahd deutlich negativ aus.

In Kapitel 4 wird eine Freilandstudie vorgestellt, in der auf einer dicht mit Ackerkratzdisteln bestandenen Brachfläche über einen Zeitraum von drei Vegetationsperioden eine inundative Behandlung der Disteln mit zwei Pathogenen durchgeführt wurde, einerseits mit Uredosporen des Rostpilzes *P. punctiformis*, andererseits mit Konidiosporen von *P. destructiva*. Um den optimalen Zeitpunkt einer Inokulation zu bestimmen (Charudattan & Dinoor 2000), wurden monatlich einfache Inokulationen mit jedem der Pilze durchgeführt, dazu jeweils eine Dreifach-Applikation jedes Pilzes, sowie eine einfache, kombinierte Applikation von *P. punctiformis* und *P. destructiva*. Kontrollen und Fungizidbehandlung dienten zum Vergleich. Im Verlauf der drei Untersuchungsjahre kam es zu einer deutlichen Reduktion der Distel durch die zunehmende Konkurrenz mit Gräsern. Zusätzlich ließ sich schon im zweiten

Jahr der Applikationen eine deutliche Reduktion der Distel-Abundanzen im Vergleich zu Fungizid-behandelten Flächen feststellen. Die Abundanz der stark geschädigten systemisch *P. punctiformis* infizierten Disteln wurde nicht von den Behandlungen, jedoch vom tatsächlichen Ausmaß lokaler Infektionen beeinflußt. *P. destructiva* infizierte Pflanzen traten im dritten Versuchsjahr deutlich häufiger auf den mit beiden Pilzen behandelten Flächen als auf Kontrollflächen auf. Die Blütenkopf-Ausbildung wurde zudem im dritten Untersuchungsjahr durch die Behandlung mit *P. punctiformis* signifikant verringert. Dies zeigt, daß die Doppelinfektion mit einem biotrophen Pilz und einem perthotrophen Pilz neben Konkurrenzeffekten mit Gräsern einen entscheidenden Beitrag zur Kontrolle der Ackerkratzdistel liefern kann.

Das fünfte Kapitel dieser Arbeit konzentriert sich auf einen weiteren wichtigen Aspekt der biologischen Kontrolle, die Wechselwirkungen zwischen verschiedenen Organismengruppen. Diese Wechselwirkungen können im Rahmen einer Applikation auftreten oder bereits natürlicherweise vorhanden sein. In zahlreichen Studien wurde der Einfluß einzelner Pathogene oder Herbivore auf Wirtspflanzen untersucht, aber nur einige neuere Studien haben mehrere Organismen einer Lebensgemeinschaft mit ihren gegenseitigen Interaktionen erfaßt (Zebitz 1990, Hatcher et al. 1994a,b, Hatcher 1995, Paine et al. 1997, Moran 1998, Friedli and Bacher 2001a,b, Hatcher and Paul 2001, Kluth et al. 2001, Kruess 2002). In dieser Untersuchung waren der pathogene Rostpilz *P. punctiformis* sowie spezialisierte herbivore Insekten an der Ackerkratzdistel Gegenstand der Forschung. Kluth et al. (2001) haben bereits gezeigt, daß Insekten durch das Vorhandensein einer systemischen Pilzinfektion mit *P. punctiformis* beeinflußt werden können. Einzelne Arten weisen Vorlieben für gesunde, andere für systemisch infizierte Disteln auf, während ein großer Teil vor allem generalistischer Herbivorer keinerlei Präferenzen zeigt. Aufbauend auf dieser Untersuchung wird im vierten Kapitel der Frage nachgegangen, inwieweit zwischen spezialisierten Herbivoren und dem Distelrost mutualistische und antagonistische Beziehungen bestehen. Es konnte gezeigt werden, daß der oligophage Blattkäfer *Cassida rubiginosa* zwar an der Verbreitung der Sporen des Rosts beteiligt war, in seiner Entwicklung dagegen bereits bei einer lokalen Infektion seiner Wirtspflanze stark eingeschränkt wurde. Im Gegensatz dazu waren die Beziehungen zwischen verschiedenen Blattlausarten und dem Rost mutualistischer Art: das Kolonie-Wachstum der Blattläuse wurde einerseits durch eine Infektion gefördert, und die Blattläuse förderten eine Verbreitung des Pilzes.

Schlußfolgerungen

Die Untersuchungen zeigen, daß die Anfälligkeit der Ackerkratzdistel gegenüber dem Rost *P. punctiformis* am höchsten bei adulten Disteln war, die kurz vor der Blüte standen oder bereits blühten und daß der Anteil anfälliger Disteln sehr hoch war, obwohl Unterschiede im Grad der Anfälligkeit bestanden. Durch Rostinfektion wurde bei gleichzeitiger mechanischer Kontrolle durch Mahd der Anteil fertiler Blüten der Ackerkratzdistel besonders stark reduziert. Unter Freilandbedingungen auf einer Brachfläche zeigte sich ein starker Konkurrenzeffekt mit der übrigen Brachvegetation, insbesondere Gräsern. Ein synergistischer Effekt bei der Reduktion der Disteln im zweiten Anwendungsjahr wurde durch eine kombinierte Inokulation mit *P. punctiformis* und *P. destructiva* erreicht. Erfolge wies ebenfalls eine langfristige Inokulation mit dem Distelrost auf: Ähnlich wie im Topfversuch mit kombinierter Mahd wurde hier die Anzahl von Blütenköpfen der Ackerkratzdistel negativ beeinflußt. Möglicherweise kann so eine Kombination der auch natürlicherweise oft in hohem Maße auftretenden lokalen Infektion mit *P. punctiformis*, gepaart mit einer Zweitinfektion durch *P. destructiva* und Konkurrenz durch die Begleitflora zur einer deutlichen Reduktion des Distelaufkommens führen.

Daß erst ältere Disteln eine hohe Anfälligkeit gegenüber einer Pilzapplikation aufweisen, reduziert den Erfolg der Biologischen Kontrolle innerhalb desselben Jahres. Wir konnten jedoch zeigen, daß durch einen hohen Grad von lokalen Infektionen der Anteil systemisch infizierter Disteln im Folgejahr gefördert wurde. Damit erscheinen langfristige Maßnahmen zur Distelreduktion mit *P. punctiformis*, möglicherweise gefördert durch eine Verbreitung des Pilzes durch spezialisierte herbivore Insekten, am erfolgversprechendsten. Als obligat biotropher Pilz ist *P. punctiformis* für einen augmentativen Einsatz mit einer relativ geringen ausgebrachten Menge von Inokulum geeignet. Eine epidemische Verbreitung ist durch die noch im selben Jahr stattfindende Vermehrung über Uredosporen gewährleistet. *P. destructiva* ist dagegen im Labor vermehrbar und wäre daher auch für einen inundativen Einsatz als Mykoherbizid geeignet (Charudattan 1988). Weitere Untersuchungen müssen zeigen, inwieweit eine langfristige Behandlung mit einer Kombination dieser beiden Pilze sich auf Distelpopulationen in der Feldfrucht auswirkt.

Influence of thistle age and phenotype on pathogen infection: comparing local and regional experiments

2

Abstract The influence of age and phenotype of creeping thistle (*Cirsium arvense*) on the susceptibility to a rust fungus infection was studied both in common garden experiments and in field trials on a broader regional scale. Manual inoculation of potted thistle seedlings and vegetatively propagated shoots with the rust *Puccinia punctiformis* resulted in age-dependent differences in susceptibility. Young thistles were almost resistant, but resistance greatly decreased as plants matured. This decrease could also be shown in field trials. Comparing the susceptibility of thistle phenotypes from ten sites around the city of Göttingen (Germany), manual inoculation lead to site-dependent infection rates (ranging from 0-8 sori per cm² leaf). Inoculation of the same phenotypes in a common-garden experiment also resulted in differential degrees of infection, but this local pattern could not be used to predict the regional, between-site pattern. These results emphasise the importance of environmental factors, taken into account only by large-scale field experiments.

Keywords Plant age, origin, fungus, biological control.

Introduction

Biocontrol of weeds with fungal pathogens has recently received much interest (e.g. Burdon et al. 1981, Frantzen 1994a, Barreto and Evans 1998, Harvey et al. 1998, Jahromi et al. 1998, Fauzi et al. 1999, Frantzen and Müller-Schärer 1999, reviewed in Charudattan and Dinoor 2000, Hurrel et al. 2001, Kluth et al. 2001). Native or introduced weed species have been screened for naturally occurring pathogenic antagonists, and their effects on the weeds and the requirements for successful inoculation have been analysed. Several studies have concentrated on examining environmental, e.g. climatic requirements of infection success (e.g. Frantzen 1994b, Jahromi et al. 1998, Fauzi et al. 1999). Nevertheless, the basis of all research on biological control with fungal pathogens should be the study of plant properties, like resistance and its dependence on host age, and their possible influence on the outcome of biocontrol programmes (Hasan 1972, Frantzen and Van der Zweerde 1994, Espiau et al. 1998, Wyss and Müller-Schärer 1999, Hurrel et al. 2001). In crop plants, the influence of plant developmental stage or phenotype on susceptibility towards fungal pathogens has been examined at length (e.g. Pretorius et al. 1988, Coutinho et al. 1994, Everts and Lacy 1996, Ojiambo et al. 1999, Chongo and Bernier 2000, Vloutoglou and Kalogerakis 2000). Although ontogenetic changes

in the relative susceptibility of plants to pathogens are common, yet variable in direction (Yarwood 1959), in biological control, where application at the time of highest susceptibility of the weed is of crucial importance for success, analyses on the effect of plant stage have only recently been undertaken (Hatcher et al. 1995, Wyss and Müller-Schärer 1999, Conner et al. 2000, Green and Bailey 2000).

Differences in susceptibility among phenotypes of the same plant species are well known for crop plants and are important for breeding of resistant cultivars. Natural host populations have likewise been found to be variable for resistance against pathogens or herbivores (Burdon 1980, Burdon and Marshall 1981, Burdon and Jarosz 1988, Jarosz and Burdon 1991, Roy 1993, Hare 2002). Differences in the susceptibility of neighbouring populations have been observed (Burdon and Thrall 1999) even within areas where particular pathogens are generally present and although their increase and spread is not hampered by physical environmental conditions (Burdon 1987).

In our study we focused on the influence of plant age and phenotype of the weed creeping thistle *Cirsium arvense* (L.) Scop. on urediniospore infection with the rust fungus *Puccinia punctiformis* (Str.) Röhl. Creeping thistle is considered one of the world's worst weeds (Holm et al. 1977) and is especially difficult to control because of its deep-growing root system and clonal reproduction (Donald 1990). The pathogen *P. punctiformis* is an autoecious rust fungus specific to *C. arvense* (Buller 1950, Frantzen 1994a). It reproduces asexually via urediniospores and sexually via teliospores. The latter infect thistle hosts via the shoot buds of horizontal roots (Van den Ende et al. 1987) and cause systemic infection in growing plants. The asexual urediniospores infect leaves and cause local lesions, where again both forms of spores are released. While systemic infection has a strong effect on thistle performance, preventing flowering and causing early death of plants, the effect of localised infection is comparably small (Buller 1950, Van den Ende et al. 1987, Kluth et al. 2002c). Nevertheless, an increase of local infections (and thereby teliospore production) is easier to achieve in the field (Kluth et al. 2001) and might lead to an increased proportion of systemic infections in the following season. Frantzen and Van der Zweerde (1994) investigated the impact of *P. punctiformis* on systemic infection of root fragments of *C. arvense* with three thistle clones in a greenhouse experiment. Our study compares research on resistance against above ground urediniospore infection of thistles as depending on plant age and phenotype, both in a common garden experiment and in field experiments on a broader regional scale. It thereby allows an estimation of the importance of environmental factors for resistance when concluding from experimental results to field conditions.

Materials and methods

Study sites

Experiments were conducted on 10 field sites within a radius of 15 km around Göttingen and at the experimental field (an old fallow) of the department of Agroecology at the University of Göttingen. The field sites used for studies were fallows and field margins of different age and size, at which with dense populations of thistles were present (Table 1). At each site, coherent horizontal thistle roots were dug from the ground, making sure that they came from one clone. From 4 cm segments of these roots, thistle plants were grown in 12 l-pots in the greenhouse (one root fragment per pot) and used for experiments at the experimental study site.

Pathogen

Urediniospores were collected from systemically infected thistles in a pasture south of Göttingen in June 1998 and 1999, respectively. Urediniospore suspensions were prepared by suspending 50 mg spore material in 100 ml water (1.1×10^5 spores / ml) and adding a drop of detergent (TWEEN® 40 Polyoxythylene sorbitan monopalmitate, Merck-Schuchardt, Germany).

Field experiment: Influence of thistle size, and origin, on infection

At each of the 10 field sites, eight pairs of thistle shoots of defined length were arbitrarily chosen and marked. Shoot length ranged from 15 cm to 120 cm in 15 cm intervals. Length of shoots was assumed to be positively correlated with shoot age. Except for the smallest thistle shoots at site 4 and 7 (15 cm), all classes of shoot length were found at each site. Half of the shoots (one of each size class) were inoculated with an urediniospore suspension in July 1998. The remaining eight control shoots were treated with water and detergent only. Each leaf of a plant's main shoot was sprayed once with a hand sprayer (Desaga, Sarstedt, NS 18,8/25 Preciso, approximately 9.0×10^3 urediniospores). After 16 days, the five lower leaves of the

Table 1 Thistle density, vegetation type and age of the fallow sites.

Clone	Site	Thistle density	Vegetation type	Approx. age
1	Göttingen NW	10-50 thistles/m²	wet grassland	> 10 years
2	Göttingen NE	< 10 thistles/m²	old fallow, bushy	> 10 years
3	Marienstein	> 50 thistles/m²	fallow, ruderal	2-5 years
4	Reinshof E	10-50 thistles/m²	field margin	2-5 years
5	Reinshof W	> 50 thistles/m²	fallow, ruderal	5-10 years
6	Weende	> 50 thistles/m²	fallow, ruderal	5-10 years
7	Reinshof S	> 50 thistles/m²	wet fallow	5-10 years
8	Rosdorf	> 50 thistles/m²	fallow, grassy	2-5 years
9	Lenglern	10-50 thistles/m²	greenland	2-5 years
10	Drakenberg	> 50 thistles/m²	fallow, ruderal	< 2years

main shoot of each inoculated and control plant were sampled and stored deep frozen. Urediniosori were counted under the binocular. The corresponding leaf length and width was measured and the number of sori per cm² was calculated. Dry weight of leaves was determined.

Common garden experiment I: Influence of thistle phenotype on infection

Thistle shoots were reared from horizontal roots of each of the 10 different origins in the greenhouse on 31st March 1998, and again a month later on the 1st May 1998. Roots of similar diameter were all cut into fragments of 3 cm length (to reduce the probability of maternal effects) and planted in standard soil in the glasshouse. Root fragments of sites 2, 6 and 8 (see Table 1) did not produce sufficient plants on one of the dates and were therefore omitted from the analysis. Eight potted plants of each date and origin were transferred outdoors after four weeks and the plant parameters, shoot length, number of shoots per pot, and number of flower heads per pot, were recorded monthly. Thistles were inoculated with an urediniospore suspension with a hand sprayer as above with 40 sprays per pot (resulting in a spore density of approximately 1.5 x 10^6 urediniospores per m² ground cover) on the evening of the 4th July. All pots were covered with plastic bags to maintain a high humidity for 48 hours. After 16 days, the three lower leaves of the three tallest shoots of each pot were sampled (= nine leaves per pot) and urediniosori counted as above. The corresponding leaf length and width was measured and dried leaves were weighed. Plants were then harvested and their dry weight determined.

Common garden experiment II: Influence of thistle age on infection

Thistle plants were reared at 14 day intervals from seeds as well as root fragments of thistles originating from a fallow south of Göttingen. Roots were dug up in May 1999 and stored in humid soil at 4° C, which kept them from shooting ahead of schedule. Seeds and root fragments were planted on the 11th April, 26th April, 10th May and the 25th of May 1999 in the greenhouse. Pots were transferred outdoors after five weeks. There were ten replicates per date for seedlings and 20 replicates per date for thistles grown from root fragments. The plant parameters shoot length, number of shoots per pot, and number of flower heads per pot were recorded monthly. Thistles were inoculated in mid July with a hand sprayer (as above) in the evening (spore density of approximately 1.5 x 10^6 urediniospores per m²) and covered with plastic bags immediately afterwards for 48 hours. We found no naturally occurring infections on the plants prior to manual inoculation. After 16 days, nine leaves of each pot were sampled as above and urediniosori counted.

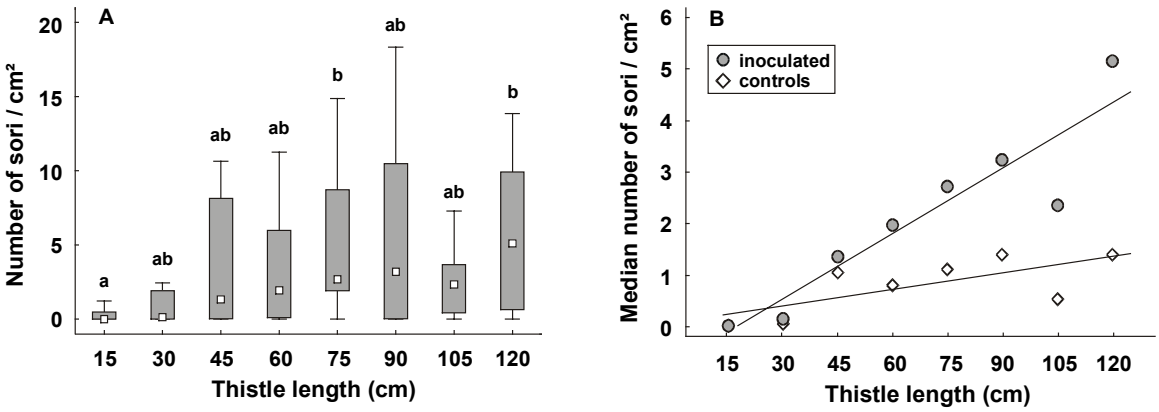

Fig. 1 Influence of shoot length of thistles on the degree of infection in the field. **A)** Influence of shoot length on the degree of infection in inoculated plants. Rank-score-tests for factorial designs, $F_{7,70}$=28; n=78; P=0.006. Box-whisker-plots with medians, 25% and 75%-quantiles and minimum and maximum values. Box-whisker-plots with different letters are significantly different. Outliers and extremes are not shown. **B)** Median numbers of sori/cm² of inoculated and control plants of different length. Inoculated: F=36.5; n=8; r^2=85.9; P<0.001; y= -0.75+0.04 x; control: F=6.38; n=8; r^2=51.5; P=0.045; y=0.06+0.01 x. Comparison of regression lines: length: F=41.9; P<0.001; intercept: F=22.1; P<0.001; slope F=14.9; P=0.002.

Statistical analyses

Data were tested for normal distribution with Shapiro Wilk's test. As part of the data did not fit the assumptions of normality, we performed rank-score tests for factorial designs (Brunner et al. 1999) with SAS Statistical Analysis Systems (2000, proc rank, proc mixed). Age and length were the factors used in age experiments, while origin and inoculation, or origin and planting date, were the factors used in the phenotype experiments. Individual treatments were compared with post-hoc Scheffé tests. Medians are given in the text. Graphs show Box-Whisker-plots with medians, 25% and 75%-quantiles, and minimum and maximum values. Outliers and extremes are not shown in Box-Whisker graphs. Different letters over box-whisker plots indicate differences at the P<0.05 level. The correlation of variables was analysed using Pearson product-moment correlations, respectively Spearman-rank correlations (site age vs. degree of infection).

Results

Influence of thistle size/age on infection severity

For the 10 field sites studied, the median number of sori that developed on the sampled leaves of inoculated thistles during the 16-day interval increased significantly with thistle length (Fig. 1 A). The median numbers of sori were close to zero in 15 cm and 30 cm small thistles, with 0.00 respectively 0.14 sori/cm², but increased to 5.1 sori/cm² in thistles that were 120 cm tall. Median numbers of sori correlated with the length of the plants in both inoculated and control plants (Fig. 1B): more sori developed on stem leaves of taller, and therefore pre-

sumably older plants than on smaller, younger plants. This correlation was highly significant especially in inoculated thistles compared to controls, where there was a weaker correlation (Fig. 1B).

Disease severity after manual inoculation in the common garden experiment differed significantly among the four planting dates in both seedlings and clonal thistles. Numbers of urediniosori were highest in the three oldest seedling groups of 14, 12 and 10 weeks of age (Fig. 2 A). Eight week old seedlings showed significantly less signs of infection. Differences in resistance were even more clearly distributed among the varying ages of clonal thistles. Oldest plants were highly susceptible with sorus numbers of 0.57 sori/cm², while 12 and 10 week old plants were less susceptible, but still developed significantly more sori than youngest plants (0.00 sori/cm², Fig. 2 B). Correlations between age and length in both clonal thistles and seedlings were highly significant, indicating that our assumptions for the field experiment were correct (Clonal thistles: F=105.75; n=98; r^2=52.40; P<0.001; y= -9.31+65.95 x; seedlings: F=44.61; n=40; r^2=54.00; P<0.001; y= -7.66+38.00 x).

Influence of thistle phenotype on infection severity

In the field experiment, inoculated and control thistles differed significantly in disease severity, this effect depending on the site (Fig. 3 A, B). Thistles of the sites 1 (Göttingen NW) and 2 (Göttingen NE) were almost resistant to infection, with median numbers of 0.00 sori/cm², whereas at other sites significantly more sori/cm² had developed (site 8: 4.72 sori/cm²; site 9: 5.45 sori/cm²; site 10: 8.28 sori/cm²). In contrast to field experiments, no significant effect of origin on disease severity could be detected in the common garden experiment. However, there was a significant interaction effect among origin and the date of

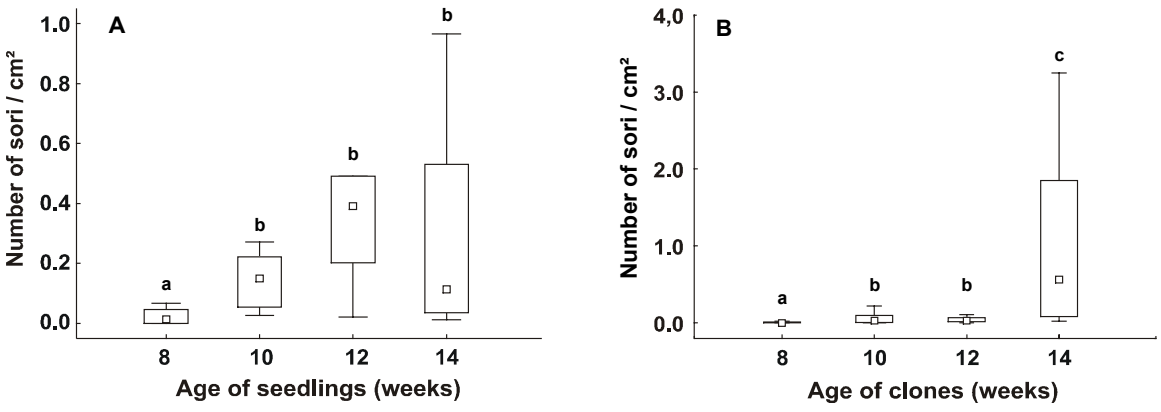

Fig. 2 Influence of the age of clones, or seedlings, on infection. Rank-score-tests for factorial designs. **A)** Seedlings: $F_{3,36}$ =9.64; n=40; P<0.001. **B)** Clones: $F_{4,93}$ =17.37; n=98; P<0.001. Box-whisker-plots with medians, 25% and 75%-quantiles and minimum and maximum values. Box-whisker-plots with different letters are significantly different. Outliers and extremes are not shown.

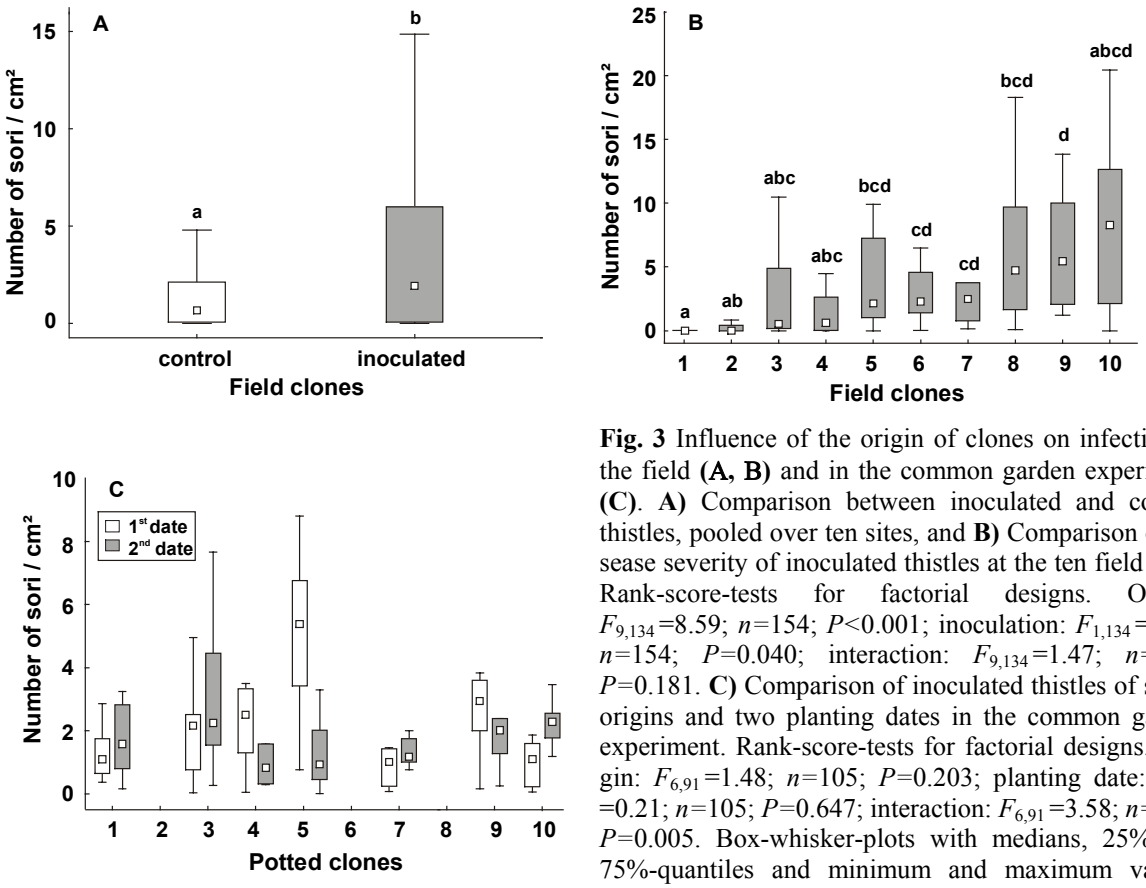

Fig. 3 Influence of the origin of clones on infection in the field **(A, B)** and in the common garden experiment **(C)**. **A)** Comparison between inoculated and control thistles, pooled over ten sites, and **B)** Comparison of disease severity of inoculated thistles at the ten field sites. Rank-score-tests for factorial designs. Origin: $F_{9,134}$=8.59; n=154; P<0.001; inoculation: $F_{1,134}$=4.34; n=154; P=0.040; interaction: $F_{9,134}$=1.47; n=154; P=0.181. **C)** Comparison of inoculated thistles of seven origins and two planting dates in the common garden experiment. Rank-score-tests for factorial designs. Origin: $F_{6,91}$=1.48; n=105; P=0.203; planting date: $F_{1,91}$=0.21; n=105; P=0.647; interaction: $F_{6,91}$=3.58; n=105; P=0.005. Box-whisker-plots with medians, 25% and 75%-quantiles and minimum and maximum values. Outliers and extremes are not shown.

planting, indicating differences in disease resistance among thistle origins, but dependent on the different planting dates (Fig. 3 C). Excluding the planting dates, differences in phenotype could be shown at the first date ($F_{6,49}$ =4.17; n=56 P=0.003). There were also differences depending on thistle origin in most of the plant parameters surveyed (Table 2).

Thistles of old fallows proved to be more resistant to localised *P. punctiformis* inoculation than thistles of very young, newly established sites (Fig. 4). No correlation could be detected between the age of sites and thistle shoot length.

Discussion

Influence of thistle age/size on infection severity

We found both field grown thistles as well as potted experimental thistles to increase in susceptibility with age respectively size. These results are consistent with observations in the field, where we found about 95% of old plants to be locally infected by the end of summer, whereas young shoots, occurring at the same time, seldom expressed signs of infection (Kluth, pers. observation). In a study testing the effect of the age of houndstongue (*Cynoglossum officinale*) on disease of the fungus *Phoma pomorum*, Conner et al. (2000)

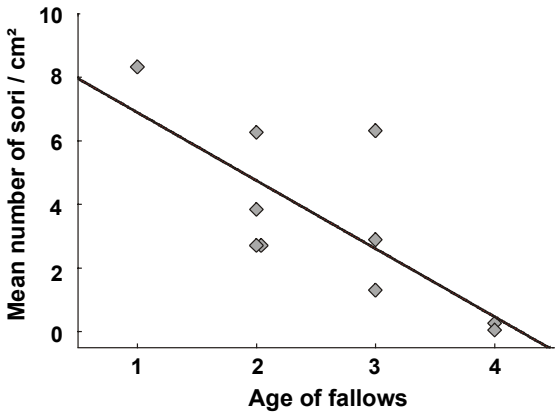

Fig. 4 Influence of the age of fallows on susceptibility of thistles towards manual *P. punctiformis* inoculation. Mean numbers of sori/cm². Spearman-rank correlation, n=10; P=0.028; R=-0.689; y=9.03-2.14 x.

found younger plants infected with the fungus to have a higher percentage of dead leaves compared to older plants. The age of leaves also strongly influenced lesion formation, with *P. pomorum* primarily attacking the older leaves of houndstongue. Green and Bailey (2000) describe a similar effect of leaf age on infection: *Alternaria cirsinoxia* was primarily pathogenic on older, senescing leaves of creeping thistle. Hatcher et al. (1995) and Wyss and Müller-Schärer (1999) each tested weeds at two stages and also found susceptibility of plants to increase with age.

Similar age effects in susceptibility are known from several crop plants. Tomato plants were shown to be susceptible to *Alternaria solani* at all growth stages, but susceptibility increased as the plants matured (Vloutoglou and Kalogerakis 2000). When inoculating sesame plants at the ages of 4, 6, 8, 10 and 12 weeks with *Alternaria sesami*, plants inoculated at 8 and 12 weeks of age were the most susceptible, whereas those inoculated at 4 weeks of age were least susceptible (Ojiambo et al. 1999). In contrast, Chongo and Bernier (2000) found both the number of lesions and disease severity to increase in lentil plants of 2 to 4 weeks of

Table 2 Effect of origin (O) and planting date (D) on plant parameters. The range of medians is given for each parameter. O x D=interaction effect; $^{(*)}$ P<0.1; * P<0.05; ** P<0.01; *** P<0.001. Rank-score test for factorial designs.

Plant parameter	Factor	F	P
Number of flower buds (ranging from 4 to 91 buds)	O	$F_{6,93}$ = 8.19	***
	D	$F_{1,93}$ = 4.35	*
	O × D	$F_{6,93}$ = 2.04	*
Shoot length (August) (ranging from 30 to 57 cm)	O	$F_{6,93}$ = 8.21	***
	D	$F_{1,93}$ = 8.87	**
	O × D	$F_{6,93}$ = 2.83	*
Number of shoots per pot (ranging from 12 to 40 shoots)	O	$F_{6,93}$ = 6.87	***
	D	$F_{1,93}$ = 48.0	***
	O × D	$F_{6,93}$ = 2.97	*
Biomass per pot (ranging from 35 to 72 g)	O	$F_{6,93}$ = 5.94	***
	D	$F_{1,93}$ = 2.89	$^{(*)}$
	O × D	$F_{6,93}$ = 2.06	$^{(*)}$

age infected with anthracnose (*Colletotrichum truncatum*), but stated a decrease in lesion number and disease severity from 4 to 8 weeks of age.

Age effects are thus well documented, and the effect of age seems to depend on the particular host-pathogen interaction investigated (Coutinho et al. 1994). But still few is known about the underlying mechanisms (Frantzen, pers. communication). The low incidence of infection in both young seedlings and clonal shoots indicates that there is a strong resistance in younger plants towards the pathogen *P. punctiformis*. This age- or stage-dependent resistance might be an effect of differences in leaf morphology, with younger leaves probably having a different microclimate due to an increased leaf wettability compared to older plants (Frantzen, pers. communication). Resistance in young plants and seedlings poses a problem to biological control, because early infection could be expected to have the highest negative impact on plant performance.

Influence of thistle phenotype on infection severity

Consistent with the differences in resistance found in the field experiment, we found differences in resistance in first date plantings of the common garden experiment. As we compared host populations not only at their site of origin, but also under the standardised conditions of a common garden experiment we can exclude the effect of site conditions. Roy (1993) assumed that the environmental influences on the probability of infection are spread among the replicated genotypes of a clone within an experiment. Therefore differences in infection at the clonal level would reflect genetic differences in susceptibility. In our study, the differences in susceptibility among thistles of different origins in the common garden experiment could therefore be expected to reflect genetic, and not just phenotypic diversity in susceptibility.

Although a direct statistical comparison among data from field and common garden experiments cannot be undertaken because of the different methods used, correlating the median disease intensities of each site in the field with those in the common garden experiment revealed no significant relationship. Although we find some statistically significant differences of Fig. 3 B (site 1 versus site 5) repeated in common garden data of the first planting date (Fig. 3 C, significant in Scheffé post hoc test of the first planting date), the comparison of both graphs (Figs 3 B and C) suggests that reactions on manual inoculation are strongly dependent on the local environment of experiments.

Host plants may be adapted to their local environment (Davelos et al. 1996). Transplanting them to a common garden with standard nutrient conditions might change their physical condition which, in turn, could influence the performance of the pathogen. A poorer physical condition may allow pathogens to easily infect plants, but may also inhibit the

development of biotrophs because of reduced nutrient supply (Davelos et al. 1996). Also, the degree of fitness differences between susceptible and resistant phenotypes may be strongly affected by the overall environmental quality (Parker 1992). This underlines the importance of the choice of environment when comparing plant performance and explains the inconsistency in the degree of resistance among our field and common garden data.

Studies involving only one or two isolates of a pathogen are unlikely to provide as detailed a picture of the resistance structure of a population as are studies involving many races of varying levels of pathogenicity (Burdon and Jarosz 1988). We used field collected urediniospores for the suspensions and presumably have yielded a mixture of several pathogen strains as we collected spores from systemically infected thistles where, in contrast to local infections with asexual urediniospores, sexual recombination had occurred.

We found thistles from old study sites to be more resistant to infection than those from more recently established sites. This may be due to high selection pressure on susceptible varieties compared to the unopposed clonal propagation of more resistant varieties in the course of time, whereas on young sites, young susceptible clones may have established due to founder effects (Burdon and Jarosz 1988) and till date have escaped extinction by pathogens.

The importance of space has been greatly overlooked in ecology (Tilman and Kareiva 1997). Pronounced spatial structuring occurs between closely adjacent populations of both host and pathogen populations. The major factors which could influence this structuring are small-scale changes in the physical environment, founder effects and localized genetic differentiation in both the host and the pathogen (Burdon and Jarosz 1988). Founder effects due to the establishment of host populations from a limited initial seed input (Burdon and Jarosz 1988) are extremely likely as a cause of the variability in resistance structure in a clonal plant like *Cirsium arvense*. Spatial structuring of host genetic variation possibly leads to pathogen adaptations to local host populations (Parker 1985). A certain degree of adaptation of the pathogen to the host population (Parker 1985; Davelos et al. 1996) may have caused the comparably high median of spore numbers / cm² on thistles of first date plantings from population 5, where urediniospores had been harvested (Fig. 3c). Paralleling resistance selection in the host, there is selection in the pathogen. Whereas selection in the pathogen is fast, the clonal growth of *C. arvense* and the low efficiency of sexual propagation (Donald 1994) reduce the chance of resistance formation in the host. This increases the chance of a stable control situation between the weed and its biological control pathogen, once established (Holt and Hochberg 1997).

In conclusion, we found significant effects of plant age on susceptibility against a rust fungus. Young plants expressed a higher resistance towards manual inoculations than old plants. Although biological control is usually aiming on early damage and early reductions of weed density, fungal infections during the growing season may still be useful, as thistle densities may become significantly reduced by a higher incidence of systemic infections in the following season. There was a great variation in the degree of infection among phenotypes both in the field and in common-garden experiments. However, the phenotype-dependent degree of infection in the common-garden experiment could not be used to predict the degree of infection of the same phenotypes growing at different field sites. Our results stress the importance of environmental effects on weed resistance towards pathogens and therefore the need of field studies on a broader regional scale.

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3 Influence of pathogen application and cutting on the performance and nutrient storage of *Cirsium arvense*

Abstract The influence of mechanical and pathogen treatments on performance and nutrient storage of the weed creeping thistle (*Cirsium arvense*) was studied over a 2-year period. We are not aware of a study evaluating the effect of a combination of cutting, and the application of the rust *Puccinia punctiformis* as a possible biocontrol organism, on thistles. We conducted a complete 2-factorial design using potted plants, with the application of the rust and cutting of thistles as the treatment factors. Treatments were applied once every year in June. Inoculation reduced above-ground biomass in the first year. First year cutting reduced the number of thistle shoots, but stimulated compensatory growth with an increased growth rate and biomass. Only a second year cutting treatment significantly reduced above-and below-ground biomass, despite of an increased growth rate. The number of flower heads was strongly reduced by both cutting and combined treatments. The proportion of fertile flower heads was increased by cutting, whereas the combination treatment strongly reduced it. Analysing storage nutrients, we did not find starch concentrations to differ among treatments. Mowing reduced sugar concentrations, but pathogen infection tended to increase concentrations, resulting in significant interaction effects. Mowing also reduced below-ground carbon concentrations, but the carbon-nitrogen ratio was not influenced. The traditional mechanical technique of cutting was shown to be effective reducing plant reproductive success and concentration of storage nutrients. Additional infection with a plant pathogen further restricted sexual reproduction. A possible synergistic effect of repeated cuttings and inoculations should improve weed control, but further studies are needed to establish *P. punctiformis* as a mycoherbicide of creeping thistle.

Keywords Biological weed control, carbon/nitrogen-ratio, creeping thistle, mechanical weed control, *Puccinia punctiformis*, rust infection, storage nutrients.

Introduction

Cirsium arvense is a persistent weed that causes problems in crop fields, grasslands and pastures as well as in fallows and nature conservation areas in temperate regions of both hemispheres (Holm et al. 1977, Donald 1994). Due to their deep-growing root system, thistles are difficult to control and the repeated use of herbicides is necessary (Donald 1994). Mechanical techniques such as repeated cutting have traditionally been applied to weaken the

thistle's capacity to regrow (reviewed in Donald 1990, Mitchell & Abernethy 1993, Mitchell & Davis 1996), but these techniques can only be applied in a small range of agricultural areas like fallow and even then may have a positive effect on thistle performance in reducing competition by other competing species (Edwards, Bourdôt & Crawley 2000). Attempts have been made using antagonist organisms to reduce thistle fitness with the help of its natural enemies. Those organisms tested have been specialist insect species (Ang et al. 1995) and plant pathogenic fungi (Frantzen 1994a, Bourdôt et al. 1995, Guske, Boyle & Schulz 1996, Harvey et al. 1998). The rust fungus *Puccinia punctiformis* is especially promising as an antagonist, because systemic infection of thistles causes early death of plants and usually prevents flowering (Buller 1950, Van den Ende, Frantzen & Timmers 1987). However, systemic infection is difficult to establish artificially in the field since infection with teliospores takes place via the root buds of emerging shoots (Frantzen & van der Zweerde 1994). Nevertheless, systemic infection has been observed after vectoring of spores by the weevil *Apion onopordi* (Friedli & Bacher 2001a). Conversely, localised infection is easily obtained by application of an urediniospore suspension to thistle leaves (Kluth, Kruess & Tscharntke 2001). This causes lesions on leaves and the ensuing urediniosori and (later in the year) teliosori spread infection. The impact of such localised infection, however, is apparently not as strong as in systemic infection, but the effects of repeated localised infection, alone or in combination with cutting treatments, on thistle reproductive fitness and nutrient storage below-ground have, to our knowledge, not been studied before.

In this study we compared the effect of two years' cutting as a mechanical control method, combined with inoculation with the biocontrol agent *P. punctiformis* and measured the effect of these treatments, singly and combined, on the development, regrowth capacity and nutrient storage of thistles.

We hypothesised that cutting thistles would, in the long term, reduce growth and nutrient storage by reducing the amount of photosynthate produced by the leaves. Inoculation with *P. punctiformis* was expected to reduce thistle fitness to a lesser extent, but in combination with cutting, evidence of synergistic negative effects was sought (Hatcher 1995, Sheppard 1996).

Materials and methods

Experimental sites and origin of plants

Experiments were conducted in a pot experiment at field conditions on fallow of the University of Göttingen, Department of Agroecology (1.5 ha). Pieces of thistle horizontal roots were recovered from the ground in spring 1998 from fallow 2 km south of Göttingen.

Coherent horizontal roots were collected to ensure that all experimental plants derived from one clone. Horizontal roots were cut into 3 cm pieces, potted in Fruhstorfer Erde potting mix (type T25, pH_{CaCl2}=5.5-6.5, N: 200-300mg/l, P_2O_5: 200-300mg/l, Industrieerdwerk Archut GMBH, Germany) and reared in the glasshouse. When approximately 15 cm tall, thistles were planted separately in 50 l plastic pots of 40 cm diameter and transferred outdoors in April 1998. Humidity of soil was controlled every other day and pots were watered when necessary. Pots were covered with hay in winter 1998/1999 to protect them from severe frosts.

Experimental design

Thistles were treated in June 1998 and 1999. A two factorial design was applied with cutting and inoculation as the treatment factors with 10 pot replicates. Treatments were as follows: 1) cutting of thistles at 30 cm height to simulate mowing, 2) inoculation of thistles with an urediniospore suspension of *P. punctiformis*, 3) both cutting and inoculation of thistles, 4) controls. Although thistles in the field are frequently mown at lower heights, the 30 cm height was used to permit infection of leaves with the fungus in the combination treatment.

The spore suspension comprised 50 mg *P. punctiformis* urediniospores per 100 ml H_2O plus one drop of detergent Tween® 40. This suspension was applied to treatments 2 and 3 with a manual atomiser (Desaga, Sarstedt, NS 18,8/25 Preciso), spraying 40 times from all sides onto each pot (3,4 ml suspension per pot, resulting in approximately 1.7 mg urediniospores per area of each pot), combined treatments being inoculated immediately after cutting. Thereafter, all treatments were covered for 48 hours with plastic bags to maintain high humidity. Weather conditions were comparably dry during the summer months of 1998 and 1999, with precipitation at most dates being less than the 30 year mean. For details see Table 1 (climatic data for Göttingen, DWD 1999, 2000).

Table 1 Climatic conditions in Göttingen from May until August 1998, and 1999, respectively. Mean temperature, deviation of the temperature from the 30-year mean, relative humidity, precipitation and the percent deviation from the 30-year mean are given[1].

Göttingen	Mean Temp. (°C)	T. Deviation (K)[2]	Rel. Humidity (%)	Precipitation (mm)	P. Deviation (%)
May 98	13.7	1.1	74.0	60.0	100.0
June 98	15.9	0.2	79.0	65.0	80.0
July 98	15.7	-1.4	79.0	45.0	71.0
August 98	16.0	-0.7	72.0	53.0	88.0
May 99	13.7	1.1	68.0	52.0	87.0
June 99	15.0	-0.7	75.0	52.0	64.0
July 99	18.4	1.3	72.0	52.0	83.0
August 99	16.5	-0.2	74.0	88.0	147.0

[1]Source: DWD 1999, 2000
[2]K=Kelvin (1 Kelvin = 1 °C)

Measurements

Samples of the lower leaves of main shoots of thistles were taken in August 1998 (5 leaves per pot, each of a different shoot) and August 1999 (10 leaves per pot, accordingly) to assess the extent of inoculation. The leaf area removed per pot was less than 1% of total leaf area per pot. Leaves were stored deep frozen until sorus numbers were counted under the stereo microscope in winter 1998 and 1999, respectively. Leaf area was estimated by calculating length x width of each leaf. The width of each leaf was measured at the widest part of the leaf, not considering the lobes. Numbers of sori were related to leaf area (sorus density). Thistle size and total number of shoots as well as the number of flower buds and fertile flower heads were recorded monthly in July and August 1998 and from Mai to August in 1999. Relative growth rate was determined as $RGR=(\ln h_2-\ln h_1)/\Delta t$ (Hunt 1989), with h_1 and h_2 being the mean height of thistles at the time of measurement in July and August of the same year, and Δt the number of days between measurements. Total above-ground dry matter was measured by the end of September 1998, after the above-ground plant parts had died. All plants were harvested in September 1999, and above- and below-ground dry matter was measured. After harvest, root matter was therefore washed and dried at 80°C for approximately two days until weight constancy.

Nutrient analysis of below-ground biomass

Dry root matter was ground up in October 1999 and stored dry. All nutrient analyses were conducted in January 2000. To gain methanol extracts of the dried root matter, 100 mg of dry matter of each sample were weighed, suspended in 5.0 ml methanol and then homogenised in a Potter–Elvehjem-glass homogeniser at 8.5°C. The suspensions were centrifuged. The residues of each sample were suspended twice in 2.5 ml methanol, centrifuging and decanting each time. Extracts were then centrifuged for 15 minutes at 4°C and the resulting clear methanol extracts decanted. These were stored at –18°C.

Total sugar content was determined with anthron reagent (Merck) following the method of van Handel (1967). 0.05 ml of methanol extracts were vaporised at 90°C, mixed with 1.0 ml of demineralised sterilised water and 6.0 ml of anthron reagent. The absorptions of these solutions were measured photometrically against the 0-value at a wave length of 630 nm. Reducing sugars were determined following the method of Somogyi (1945) and Nelson (1944). 0.1 ml of methanol extracts were vaporised at 90°C, the residues mixed with 1.0 ml of demineralised sterilised water and 1.0 ml of Somogyi-reagent. The solutions were then boiled in a water bath for 20 minutes, mixed with 2.0 ml of Nelson's reagent and centrifuged. Residues were measured photometrically at 530 nm wave length. To determine sucrose

content (Cardini & Leloir 1955, Roe 1934), 0.1 ml of methanol extracts were vaporised at 90°C, residues mixed with 0.15 ml NaOH and boiled in a water bath for 10 minutes. 1.0 ml H_2O_{demin}, 3.6 ml Roe-HCl and 1.2 ml Roe-resorcin were added and the solutions heated to 80°C. The absorption of solutions was measured photometrically at 490 nm wave length.

Total amount of starch was determined using colorimetric analysis following the methods of Shannon (1968) and Krisman (1976). Of every sample, dried ground root material was suspended in 10 ml $CaCl_2$ and boiled in a water bath for 60 minutes. The samples were then centrifuged, and clear fractions mixed with iodine solution. The total starch content was determined photometrically at 635 nm and 540 nm wave length.

For C/N analysis, 3 to 4 mg of dried ground root material were weighed in zinc trays and the C/N ratio was determined with a C/N analyser (NA 1500 N Fisons Instruments Rodano).

Statistical analysis

Raw data were tested for normal distribution with Shapiro Wilk's test (Shapiro & Wilk 1968). As data did not fit the assumptions of normality and transformation did not improve the distribution, rank-score tests for factorial designs with and without repeated measures were performed (Brunner, Munzel & Puri 1999). Cutting and infection were the factors used and year was the repetition factor. Additive effects of factors can be expected when both factors used are significant, while their interaction term is not significant. If the interaction effect is significant, too, then the combined effect is synergistic or inhibitory, depending on whether the combination of treatments has an increased or decreased effect. Post-hoc Scheffé tests were performed to compare the effects of individual treatments. Medians are given in the text. Graphs show box-whisker-plots with medians, 25% and 75% -quartiles, and minimum and maximum values. Outliers and extremes are not shown in box-whisker graphs. Correlations between variables were analysed using Spearman-rank correlations for non-parametric data. Thistles of one pot were damaged by voles in 1998, this pot was excluded from analysis.

Results

Effect of treatment factors on sorus density

All three factors applied (cutting, inoculation and year) significantly increased sorus density on thistle leaves (Fig. 1A, Table 2). Artificial inoculation increased infection on treated plants in both years. The effect was more obvious in the first year (Fig. 1A) where median numbers of sori were three times higher in inoculated treatments than in the untreated controls. In 1999, the amount of infection was higher overall compared to 1998. In both years, combined

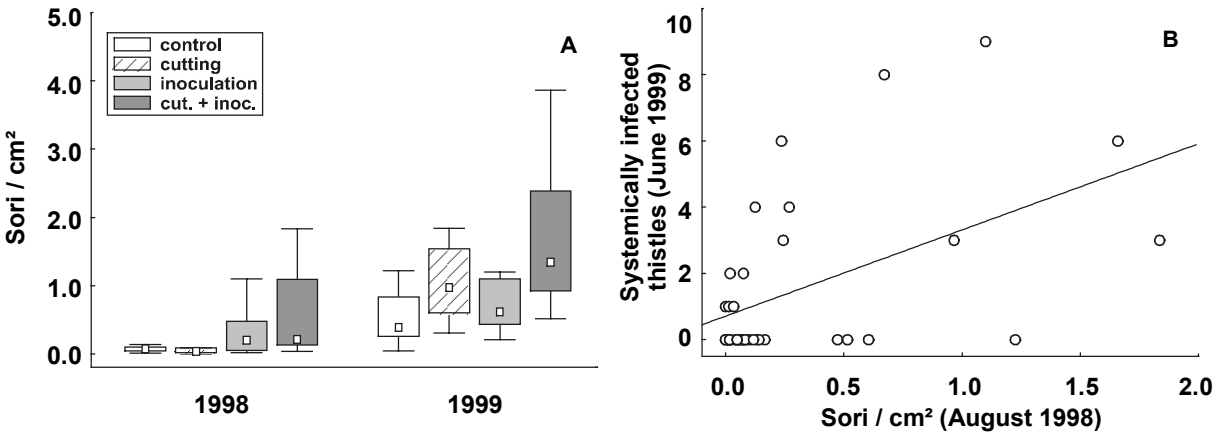

Fig. 1 Effects of inoculation of thistles with an urediniospore suspension on the local and systemic infection rate. **A)** Number of sori per cm² of leaf area on controls, cut, inoculated, and cut and inoculated thistles in 1998 and 1999. Rank-score tests for factorial designs with repeated measures. Box-whisker-plots with medians, 25% and 75%-quartiles and minimum and maximum values. Outliers and extremes are not shown. For detailed statistics see Table 2. **B)** Influence of density of sori in August 1998 on number of systemically infected thistle shoots in June 1999. Spearman-rank-correlation. n=36; R=0.379; t (N-2)=2.393; P=0.022; y=0.681+2.862 x.

treatments with inoculation and cutting had the highest impact on sorus formation (Fig. 1A). Fifty-four systemically fungus-infected thistle shoots had developed in 15 of the pots by June 1999 (3.34% of all shoots). The number of sori per leaf area in 1998 correlated significantly positive with the number of systemically infected shoots in June 1999 (Fig. 1B). The number of systemically fungus-infected shoots in June 1999 also correlated positively with the number of sori/cm² developing by August 1999 (Spearman rank correlation, n=35; R=0.329; t(n-2)=2.004; P=0.053; y=0.860+0.363 x).

Impact of cutting and infection on number and growth of shoots

An overall median of 25 shoots per pot had grown from the pieces of horizontal roots by August of the first year, this number increased to 54 shoots in the second year. Numbers of shoots differed significantly among the four treatments in August 1998, when shoot number was significantly reduced by cutting from a median of 44 shoots in controls to 15 shoots in cut treatments (rank-score test, n=39, inoculation: not significant (n.s.); cutting: $F_{1,35}$ =5.20; P=0.029; interaction: n.s.). This difference was compensated for in the second year and did not recur in August 1999.

Cutting of shoots in June of both years resulted, as expected, in a reduction in shoot height after one month. Despite a significant increase in relative shoot growth rate in cut treatments of both years (Fig. 2, Table 2), in 1998, the difference in height was only compensated for by August. In 1999, increased shoot growth could not compensate loss in shoot height anymore, cut plants remained significantly smaller (81 cm and 78 cm in cut or combined treatments,

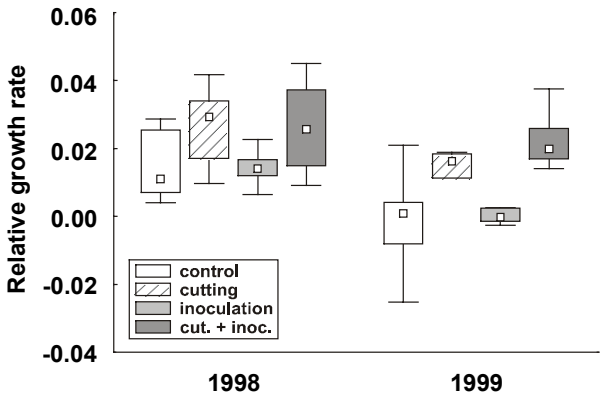

Fig. 2 Relative shoot growth rate of the five tallest shoots per pot in 1998 and 1999 after treatments in June of each year. Rank-score tests for factorial designs with repeated measures. Box-whisker-plots with medians, 25% and 75%-quartiles and minimum and maximum values. Outliers and extremes are not shown. For detailed statistics see Table 2.

respectively) than controls (132 cm) or inoculated thistles (131 cm). Infection did not significantly influence either number of shoots, growth rate or height in both years.

Impact of cutting and infection on above- and below-ground biomass

In the first year, above-ground biomass was significantly increased by cutting (Fig. 3A, Table 2). Compared to the elevated relative growth rate due to cut treatments (Fig. 2, Table 2), infection did not affect growth rate and thereby lead to a significant reduction in above-ground biomass (rank-score test with 1998 data only, n=39; inoculation: $F_{1,35}$ =5.41; P=0.026; cutting: $F_{1,35}$ =15.59; P<0.001; interaction: n.s.). In 1999, cutting alone was the significant factor reducing above-ground biomass (Fig. 3A; rank-score test with 1999 data only, n=39; inoculation: n.s.; cutting: $F_{1,35}$ =16.36; P<0.001; interaction: n.s.), losses in biomass due to cutting not being compensated for anymore by the increased relative growth rate.

Below-ground biomass (1999) was also significantly reduced by cutting (Fig. 3B), whereas the effect of inoculation was not significant. The ratio of above- to below-ground biomass (shoot-root ratio) was not influenced by either cutting or inoculation.

Table 2 The effect cutting and inoculation, singly and combined, on number of sori per cm² of leaf area, relative shoot growth rate and above-ground biomass in 1998 and 1999 (see Figs 1A, 2 and 3A. Rank-score tests for factorial designs with repeated measures.

Effect	Sori/cm²		Relative shoot growth rate		Above-ground biomass	
	d.f.	*F*	d.f.	*F*	d.f.	*F*
Cutting	1	5.56 *	1	31.94 ***	1	0.06
Inoculation	1	5.84 *	1	0.51	1	1.36
Year	1	39.66 ***	1	13.08 ***	1	38.33 ***
C*I	1	0.07	1	1.45	1	0.88
C*Y	1	1.36	1	0.67	1	11.57 **
I*Y	1	0.38	1	0.66	1	0.49
C*I*Y	1	0.33	1	1.74	1	0.38
Error	67		72		74	

* P< 0.05; ** P< 0.01; *** P< 0.001; otherwise P>0.05.

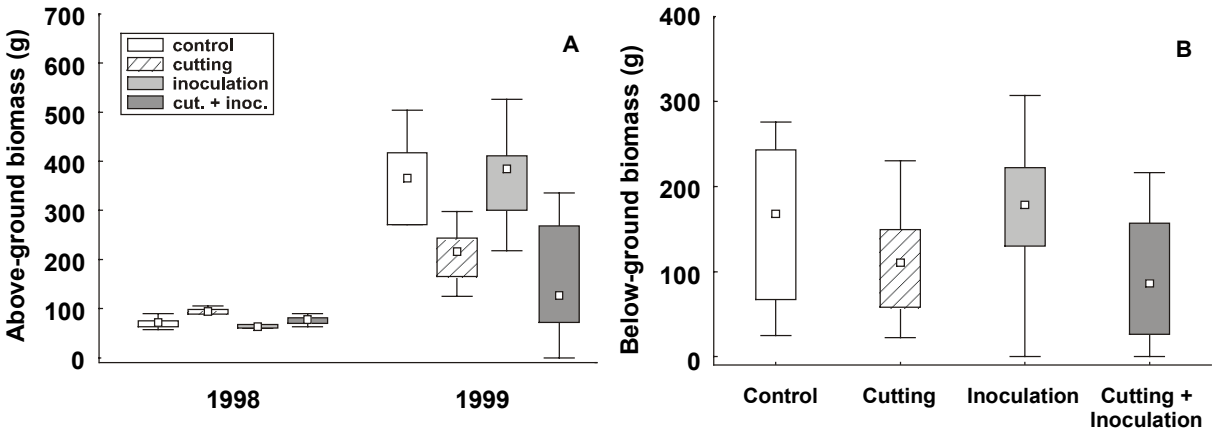

Fig. 3 Above- and below-ground biomass of controls, cut, inoculated, and cut and inoculated thistles. **A)** Above-ground biomass in 1998 and 1999. Rank-score tests for factorial designs with repeated measures. Box-whisker-plots with medians, 25% and 75%-quartiles and minimum and maximum values. Outliers and extremes are not shown. For detailed statistics see Table 2. **B)** Below-ground biomass in 1999. Rank-score tests for factorial designs. Infection: $F_{1,35}$ =0.04; P=0.843; cutting: $F_{1,35}$ =5.72; P=0.022; interactions: $F_{1,35}$ =0.24; P=0.630. Box-whisker-plots with medians, 25% and 75%-quartiles and minimum and maximum values. Outliers and extremes are not shown.

Impact of cutting and infection on flowering

Plants flowered earlier in 1999 than 1998. In August 1998, the proportion of blooming flower heads was 6% of total flower heads per pot (four blooming flower heads per pot), the proportion rose to 43% blooming flower heads per pot in August 1999 (66 flower heads per pot. In 1998, the numbers of flower heads per pot tended to be higher in cutting and inoculation treatments of thistles compared to controls, although differences were not significant (Fig. 4A, Table 3). In 1999, repeated cutting strongly reduced the number of fertile shoots and flower heads throughout the year from 716 flower heads in controls to 56 flower heads in cut and 73 flower heads in combined treatments, respectively (Fig. 4A, Table 3). By August 1999, plant reproductive fitness was significantly influenced by both cutting and

Table 3 The effect cutting and inoculation, singly and combined, on the number of flower heads and the proportion of fertile flower heads of thistles in 1998 and 1999 (see Figs 4A and B). Rank-score tests for factorial designs with repeated measures.

Effect	Number of flower heads		Fertile flower heads (%)	
	d.f.	*F*	d.f.	*F*
Cutting	1	7.98 **	1	3.35 (*)
Inoculation	1	0.24	1	1.52
Year	1	8.08 **	1	50.65 ***
C*I	1	1.11	1	3.22 (*)
C*Y	1	12.85 ***	1	2.98 (*)
I*Y	1	0.03	1	7.15 *
C*I*Y	1	0.67	1	7.00 *
Error	74		74	

(*) P<0.1; * P<0.05; ** P<0.01; *** P<0.001; otherwise P>0.1.

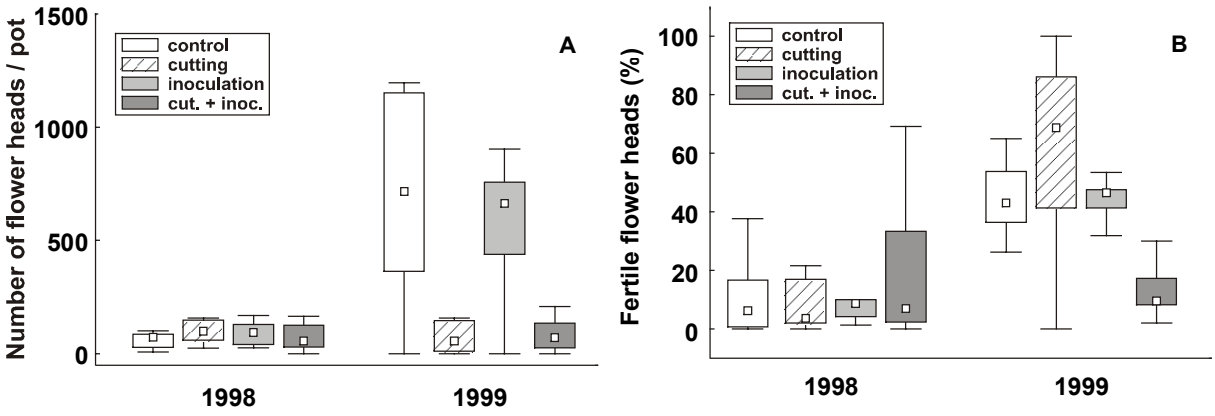

Fig. 4. Number of flower heads per pot and proportion of fertile flower heads of controls, cut, inoculated, and cut and inoculated thistles. **A)** Number of flower heads per pot in 1998 and 1999. Rank-score tests for factorial designs with repeated measures. Box-whisker-plots with medians, 25% and 75%-quartiles and minimum and maximum values. Outliers and extremes are not shown. For detailed statistics see Table 3. **B)** Proportion of fertile flower heads in 1998 and 1999. Rank-score tests for factorial designs with repeated measures. Box-whisker-plots with medians, 25% and 75%-quartiles and minimum and maximum values. Outliers and extremes are not shown. For detailed statistics see Table 3.

inoculation. The proportion of fertile, blooming flower heads was increased by cutting from 43% in controls to 69%, whereas the combination of inoculation and cutting had an inhibitory effect and drastically reduced the proportion of fertile flower heads on all flower heads to 9% (rank-score test 1999 data only: n=34; inoculation: $F_{1,30}$ =11.09; P=0.002; cutting: $F_{1,30}$ =3.22; P=0.083; interactions: $F_{1,30}$ =14.99; P<0.001; Fig. 4B).

Impact of cutting and infection on nutrient storage

Starch and sugar concentrations were analysed and determined the carbon-nitrogen ratio in below-ground biomass. Starch was only found in low concentrations with a median concentration of 0.93 mg/g dry matter and concentrations were not different among the treatments.

Median of sucrose concentration was 5.85 mg/g dry matter. Cutting marginally reduced sucrose concentrations in single cutting treatments, whereas the significant interaction between cutting and inoculation reflected a synergistic effect of combined treatments on sucrose concentrations that evened out reductions by cutting (Fig. 5A).

Changes in concentrations of reducing sugars paralleled those in sucrose concentrations. Concentrations in cut treatments were lower while combined treatments (cutting, inoculation) resulted in concentrations similar to those of controls (rank-score test, n=37; inoculation: n.s.; cutting: n.s.; interactions: $F_{1,33}$ =6.73; P=0.016).

Concentrations in total sugar content were significantly reduced by cutting. As with sucrose, the combination of cutting and inoculation resulted in similar concentrations as in controls, inoculation having a stimulatory effect on cutting (rank-score test, n=37; inoculation: n.s.; cutting: $F_{1,33}$ =4.19; P=0.049; interactions: $F_{1,33}$ =9.71; P=0.004).

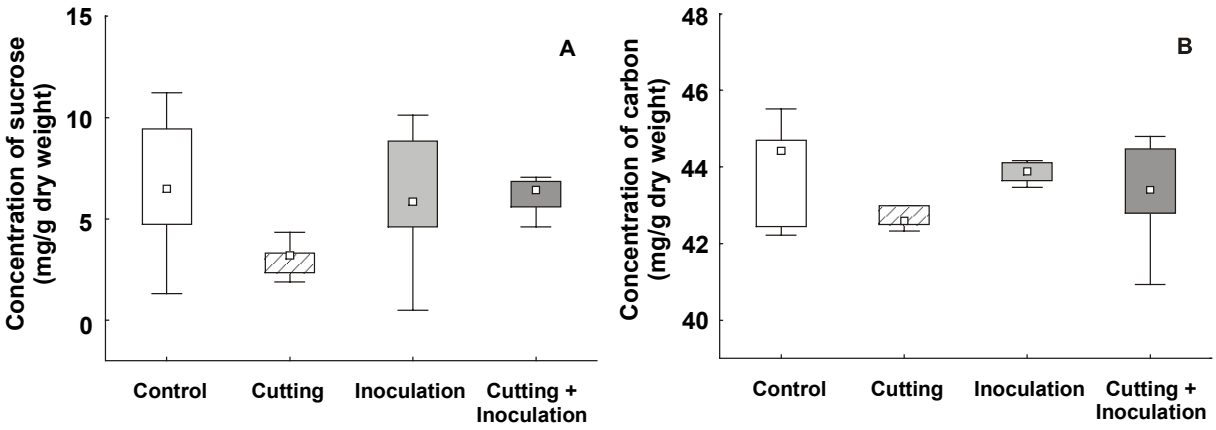

Fig. 5. Concentration of sucrose and carbon in below-ground biomass of controls, cut, inoculated, and cut and inoculated thistles. **A)** Concentration of sucrose in below-ground biomass in 1999. Rank-score tests for factorial designs. Infection: $F_{1,33}$ =2.96; P=0.096; cutting: $F_{1,33}$ =3.77; P=0.062; interactions: $F_{1,33}$ =4.32; P=0.046. Box-whisker-plots with medians, 25% and 75%-quartiles and minimum and maximum values. Outliers and extremes are not shown. **B)** Concentration of carbon in below-ground biomass in 1999. Rank-score tests for factorial designs. Infection: $F_{1,32}$ =0.14; P=0.713; cutting: $F_{1,32}$ =2.94; P=0.096; interactions: $F_{1,32}$ =0.22; P=0.639 Box-whisker-plots with medians, 25% and 75%-quartiles and minimum and maximum values. Outliers and extremes are not shown.

No differences in nitrogen concentrations among the treatments were found, but carbon concentrations were significantly reduced by cutting at the p<0.1 level (Fig. 5B). C/N-ratio, nevertheless, was not influenced by any of the treatments.

Discussion

Interpretation of cutting effects

The median numbers of sori on thistle leaves were not only increased by inoculation, but also by cutting treatments. The abiotic stress of cutting treatments on thistles may have predisposed them to infection (de Nooij 1992) and could have increased their susceptibility towards *P. punctiformis*. Inoculum of *P. punctiformis* was abundant on the small proportion of second year systemically infected thistle shoots, which presumably occurred due to natural root bud infection after first year inoculations. Spores might have spread from these systemically infected thistles to adjacent plants by either wind or insect transmission (Kluth, Kruess & Tscharntke 2002a).

Shoot number and height were mostly influenced by cutting treatments. The first year reductions of shoot numbers due to cutting might be the result of a root system little developed in the first year. Only few root buds existed to produce new shoots, rather, resources were allocated to existing shoots, resulting in their compensatory growth. With a more extensive root system in the second year, and with apical dominance being broken by

cutting, new shoots could develop from the numerous buds on horizontal roots, but compared to the first year of treatments, this obviously occurred at the expense of overall shoot height.

The overcompensation occurring in above-ground biomass of thistles due to cutting in the first year of study by the development of numerous axillary shoots (Järemo, Nilsson & Tuomi 1996) was short-termed and reversed in the second year, when cutting significantly reduced above-ground, below-ground and total biomass. Tissue loss by cutting was only partially compensated for by increased growth rates in the second year of treatments. Partial compensation took place, when the treated plants had an increased growth rate after defoliation, but did not compensate for complete tissue loss (Belsky 1986). Likewise, Stoll, Egli & Schmid (1998) observed mowing to strongly reduce the below-ground biomass of persisting old rhizomes of *Solidago altissima* and to further reduce clonal growth in the following year.

Paige & Whitham (1987) and Paige (1999) describe overcompensation in reproductive fitness of Scarlet gilia following mammalian herbivory or simulated herbivory. Although this overcompensation was questioned by Bergelson, Juenger & Crawley (1996), the reduced competition for substrates among developing reproductive organs after defoliation may increase the fertility of the remaining flower heads (McNaughton 1983). These beneficial effects of defoliation or pathogen infection may nevertheless only take place if the damage is short termed. Once storage reserves are depleted, the ability of compensation should be greatly restrained.

As a response to defoliation, plants are known to express a range of morphological mechanisms including changes in leaf-area ratio, in plant architecture and in root development (Prins & Verkaar 1992). Also, an improved plant water status due to an increase in root-shoot ratio after cutting tends to promote compensatory growth. Physiological mechanisms include a stimulation of photosynthetic rate, a redistribution of carbohydrates and mineral nutrients, and delayed plant senescence due to an increase in stomatal conductance (McNaughton 1983, Walters 1985, Prins & Verkaar 1992). The allocation of more resources to above-ground regrowth is a common and widespread reaction of plants to herbivory (or defoliation). As well as an enhanced net assimilation rate it may lead to the successful replacement of leaf tissue. Otzen & Koridon (1970), studying seasonal changes of organic food reserves in the roots of *C. arvense*, found root dry matter to strikingly parallel the amount of carbohydrates available in roots. The strong reductions in root biomass in our study were likewise paralleled by reduced sucrose concentrations in roots. Nevertheless, Heichel & Turner (1983) found much increased rates of photosynthesis in the remaining leaves of partially defoliated red maple and

red oak. Trees that had been defoliated 50% replaced very little leaf area, but reached 75% of photosynthetic capacity of control trees due to enhanced assimilation rates. However, this compensatory response depends largely on nitrogen availability (Prins & Verkaar 1992). The translocation of carbohydrates and nitrogen compounds from the roots to the leaves after defoliation can occur at rates higher than in control conditions (Prins & Verkaar 1992). In *Trifolium subterraneum* and *Trifolium repens* for example, a considerable amount of nitrogen present in storage organs was mobilised as soluble protein and directed to the newly formed leaves after defoliation (Culvenor, Davidson & Simpson 1989, Marriot & Haystead 1990, Culvenor & Simpson 1991). However, in our study, root nutrient analyses after harvest showed no significant differences in nitrogen concentrations between defoliated plants and controls. Likewise, Egli & Schmidt (2000), in a study testing the influence of a yearly mowing treatment on seasonal dynamics of biomass and nitrogen in *Solidago altissima*, did not find any significant effect of mowing on the nitrogen allocation to rhizomes in *S. altissima*.

In some studies applying cutting amongst other treatments on *C. arvense*, cutting was applied several times per year (reviewed in Donald 1990, Beck & Sebastian 2000). In contrast to that, in our study inoculation treatments and cutting, as a traditional means to reduce weed fitness, were applied only once per season, but over two consecutive years. Cutting nevertheless strongly reduced overall fitness of thistles in pots after a second year treatment, although, in the first year, cutting increased some fitness parameters. Repeated cuttings within one year were shown to control up to 85% of thistles on one site in a study by Beck and Sebastian (2000), but failed to control it at another.

Interpretation of inoculation effects

Hatcher (1996) analysed the implications of herbivory by a chrysomelid beetle and the rust fungus *Uromyces rumicis* on growth and overwintering of two *Rumex* species and, as in this experiment, found little effect of pathogen infection alone on shoot and root weight of *R. obtusifolius*.

Because a diseased leaf typically has a lowered rate of photosynthesis, an additional sink for assimilate in the form of the pathogen, and an increased rate of respiration, less translocation of carbohydrates from the diseased leaf to the roots would be expected (Farrar 1992). Ayres (1991) also reports that pathogens such as rust or powdery mildew fungi reduce the rate of net photosynthesis at the site of infection. In our study, there were no reduced concentrations of either sucrose or reducing sugars, starch (which has been reported to be rather rare as a storage nutrient in thistles, Otzen & Koridon 1970), nor reduced concentrations of carbon in the below-ground biomass of thistles after inoculation. Thus, a possible

stimulation of photosynthesis in uninfected leaves (reviewed by Walters 1985, Ayres 1991) might have compensated for the impact of local infection on assimilation rates. Also, inhibition of net photosynthesis in infected leaves may be compensated by an increase in leaf-area ratio (Ayres 1991). As opposed to locally infected thistles, losses in photosynthesis in systemically *P. punctiformis*-infected thistles may be stronger, no healthy leaves being there for compensation. Walters (1995), discussing the comparability of compensatory physiological plant reactions towards defoliation and pathogen infection, found the hormonal balance of plants might be changed in both treatments. This hormonal change might also explain a possible compensatory photosynthetic activity. A similar increased resource allocation to above-ground regrowth as after defoliation has been described by Ayres (1991) to occur after infection by fungi. Nevertheless, differences between defoliation treatments and inoculation experiments must be taken into account (Walters 1995). Defoliation allows quick recovery after the cutting event, whereas pathogens colonize the plant over a longer period and may alter host metabolism and physiology in various ways (Walters 1985).

Interpretation of combined effects of cutting and inoculation

Compared to above and below-ground biomass, plant reproductive fitness was influenced by both cutting and inoculation. While compensation tended to increase the number of flower heads in the cut and inoculated thistles in the first year, notably the combination of treatments reduced the numbers of flower heads in 1998, as well as the percentage of fertile flower heads in 1999. Although the importance of sexual reproduction in thistles is low compared to vegetative propagation (Heimann & Cussans 1996), seedling recruitment is one of the most important forces in maintaining a high genetic diversity in clonal populations (Watkinson & Powell 1993). Due to their lack of competitiveness, thistle seedling establishment is likely only in places with a high proportion of bare ground, like ploughed or disturbed areas (Heimann & Cussans 1996).

Comparability of experimental results with field situations

Potted thistles were used in this experiment to be able to harvest and analyse thistle roots. The results of this study can nevertheless be expected to be comparable to field conditions. In the field, thistles are additionally subject to competition with other plants. Competition has well known adverse effect on the survival of weeds (Sheppard, Smyth & Swirepik 2001, Weiner, Griepentrog & Kristensen 2001). As thistles have been shown to be extremely sensitive to competition especially by grasses (Holm et al. 1977, Kluth et al. 2002d), the effects of cutting

and inoculation can possibly be expected to be even more severe than in experiments with potted plants.

Thistles of one single genotype have been used for the experiments to allow a better comparison of the treatment effects. The negative effect of cutting on thistle fitness can be expected to apply also for other genotypes, as several other studies have also reported negative effects of cuttings on thistles (reviewed in Donald 1990, Mitchell & Abernethy 1993, Beck & Sebastian 2000). In other experiments with thistles from different clones we could observe that artificial inoculations with urediniospore suspensions of *P. punctiformis* readily resulted in local infection (Kluth et al. 2001, 2002a,b). Kluth et al. (2002b) were able to show differences in the susceptibility of thistle phenotypes from different origins. Only one out of the ten field clones studied did not show signs of infection in the field, but under optimised conditions in semi-field experiments with the same (potted) clone, this phenotype likewise developed sori. It can therefore be expected that infection would take place in thistles under field conditions as well, although further studies would be helpful, developing and optimising mycoherbicide formulations that improve independence from the thistle phenotype, weather and humidity conditions.

In our experiment, thistles were cut at 30 cm height, whereas in field situations, thistles might be cut much lower at about 10 to 15 cm height. This would reduce the surface of thistles that could receive inoculum in case of a mycoherbicide application, but young thistles' lower leaves as well as the remaining basal shoots of older thistles could still receive inoculum. Further studies might also test an inoculation prior to mowing, which possibly allows the fungus to attack host tissue of the whole plant, whereupon the above-ground biomass available for photosynthesis could be drastically reduced by mowing.

Conclusions

To increase stress on thistles the effect of cutting and inoculation was tested alone and in combination. The impact of inoculation alone was small, but pathogen applications combined with cutting reduced the proportion of fertile flower heads significantly. Second year effects of treatments on fitness parameters were generally more extreme than in the first year, indicating that long-term treatments with a combination of cutting and inoculation might result in additive or possibly synergistic effects compared to single treatments. As the single cutting treatment already resulted in severe reductions in fertility and thistle biomass after two years of study, repeated cuttings could be expected to exert even more pressure on thistles. The importance of longer term treatments could also be seen in the increased rate of systemically infected thistle shoots that developed in the second year of study due to an

increased density of inoculum the preceding year. These systemically infected shoots were severely damaged and died before flowering (Buller 1950, Van den Ende, Frantzen & Timmers 1987). Because a mycoherbicide containing *P. punctiformis* is not yet commercially available for application, field recommendations must be preliminary. Further studies will be needed developing *P. punctiformis* as a mycoherbicide and evaluate it in combination with cutting at field conditions. Still the results of our study are encouraging in that a combination of inoculation and cutting might reduce thistle populations in a sustainable way.

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4 Two pathogenic fungi as biocontrol agents of *Cirsium arvense* on successional fallow

Abstract Creeping thistle, *Cirsium arvense,* is a troublesome weed that is difficult to control without repeated herbicide use. Plant pathogens may be effective biocontrol organisms, and we applied spores of two fungi, the perthotroph *Phoma destructiva* and the biotroph *Puccinia punctiformis*, for three subsequent years, alone, combined and at different dates, to reduce the performance of *C. arvense* populations on a successional fallow.

C. arvense suffered from strong competition with the co-occurring fallow vegetation, and decreased from approximately 60% cover to approximately 5% cover within three years. The thistle to grass cover ratio was significantly reduced during the three years of study. Single inoculations of each of the two fungi had no significant effect on the number and height of thistles, but combined inoculations with both *P. punctiformis* and *P. destructiva* reduced thistle abundance in the second year, compared to a fungicide treatment. The number of systemically *P. punctiformis* infected thistles was not influenced by treatments, but correlated significantly with the degree of local infection. Infection with *P. destructiva* in combined inoculations was enhanced in the third year. Localised infection with *P. punctiformis* on thistle leaves correlated positively with percent systemic rust-infection in the following season. Further, localised infection significantly reduced flower bud production after three years.

In conclusion, results showed a great susceptibility of *C. arvense* populations to competition during fallow succession, and in addition, negative effects of two pathogens and synergistic negative effects of combined pathogen applications, indicating benefits of long-term treatments with more than one biocontrol agent.

Keywords *Cirsium arvense, Puccinia punctiformis, Phoma destructiva,* competition, long-term treatments, synergistic effects.

Introduction

The biological control of weeds with fungal plant pathogens has received much interest in the last three decades (Wilson 1969, Charudattan and Walker 1982, Wapshere et al. 1989, Hasan and Ayres 1990, TeBeest et al. 1992, Frantzen 1994a, Charudattan and Dinoor 2000). Pathogens for the biological control of *C. arvense* have been studied by various groups (French et al. 1987, Van den Ende et al. 1987, Watson and Keogh 1980, Brosten and Sands

1986, French and Lightfield 1990, Frantzen 1994a, Frantzen and Van der Zweerde 1994, Bourdot et al. 1995, Harvey et al. 1998, Green and Bailey 2000, Friedli and Bacher 2001b, Green et al. 2001, Hurrel et al. 2001, Kluth et al. 2001, 2002 b,c). Brosten and Sands (1986) conducted field trials with *Sclerotinia sclerotiorum* (Lib.) de Bary and found 20 to 80% of thistle shoots killed after applications. Still, *S. sclerotiorum* is not specific to creeping thistle, and also infects various other plants (Bourdot et al. 1995). *Alternaria cirsinoxia* Simmonds and Mortensen is another recently described species found to be endemic on *C. arvense* in Sascatchewan and Montana (Simmonds and Mortensen 1997). It causes severe foliar necrosis on thistle leaves (Green et al., 2001). Guske (1995) analysed the fungi present within the tissue of *C. arvense* in Germany. Among several fungi within thistle tissue, the necrotroph *Phoma destructiva* Plowr. (Sphaeropsidales) and the biotroph *Puccinia punctiformis* (Str.) Röhl (Uredinales) appeared to be promising candidates as biological control organisms for creeping thistle. For three subsequent years, we tested the impact of these two fungi on fitness and both sexual and asexual reproduction of *C. arvense* at field conditions on fallow.

Contrary to *A. cirsinoxia*, the rust *P. punctiformis* is a widely distributed pathogen that does not perform host alternation and completes its life cycle on *C. arvense* (Buller 1950). Former reports of *P. punctiformis* also infecting *Taraxacum officinale* (De Bary 1863, Wulf 1981) could not be confirmed in host specificity screenings by Guske (1995). It would therefore be an ideal candidate for the biological control of creeping thistle.

Research on the biological control of *C. arvense* with *P. punctiformis* has been undertaken by various groups (French et al. 1987, Van den Ende et al. 1987, Watson and Keogh 1980, French and Lightfield 1990, Frantzen 1994a, Frantzen and Van der Zweerde 1994, Friedli and Bacher 2001b, Kluth et al. 2001, 2002 b,c). Most studies applying *P. punctiformis* as a biocontrol organism for creeping thistle have by now tested the biotic and abiotic conditions necessary for inoculation success and the impact of *P. punctiformis* on creeping thistle at greenhouse conditions or in experiments with potted plants (Menzies 1953, Van den Ende et al. 1987, Watson and Keogh 1980, French and Lightfield 1990, Frantzen 1994b, Frantzen and Van der Zweerde 1994, Friedli and Bacher 2001b, Kluth et al. 2002c). However, artificially established stands of *C. arvense* may be more easily controlled than natural stands (Donald 1990). To our knowledge, the actual impact of artificial inoculation with *P. punctiformis* on thistles at field conditions has not been studied yet.

Few, and recent, studies have tested *P. destructiva* as a further potential biocontrol organism for *C. arvense* (Guske 1995, Guske et al. 1996, Kruess 2002). This necrotrophic fungal pathogen was isolated from chlorotic shoots of *C. arvense* from several places in

Germany (Guske 1995, Kruess 2002). Conidiospores of *P. destructiva* induce systemic infection in thistles which can reduce flowering and seed set of thistles (Kruess 2002, unpublished data). *P. destructiva* has been formerly described as a pathogen causing fruit rot and leaf and stem blight on tomatoes (Jones at al., 1991). Nevertheless, the thistle strain of *P. destructiva* used in this experiment and strains from different locations throughout Germany did not result in infections of tomato or other thistle species (*Cirsium vulgare, Carduus nutans*, Kruess, unpublished data).

We performed a test series over three consecutive years applying spore suspensions of the rust fungus, *P. punctiformis,* and *P. destructiva* to find out if artificial inoculation with spore suspensions of *P. punctiformis* or *P. destructiva* (1) increases the infection of thistles, (2) influences the proportion of infected thistles in the following season, (3) leads to a decreased number and fitness of thistles.

Materials and methods

Study site

The experiment was conducted at the experimental site Reinshof of the University of Göttingen (Lower Saxony, Germany) from 1998 to 2000. The site Reinshof (150m a.s.l.) is situated in the valley of the river Leine 2 km south of Göttingen. The soil at the site consists of fluvial clay soils. The long-year mean precipitation is 635 ml (May-June 203 ml, May-September 310 ml), long-year mean temperature is 8.7°C (May-July 15.3°C, May-September 15.2°C). The overall relative humidity from Mai to August 1998-2000 ranged between 68 and 81%, but was lower due to the warmer temperatures in 1999 compared to 1998 and 2000. Temperatures at Göttingen in 1998 and 2000 tended to be lower than the long-year temperature mean, while precipitation was slightly lower than the long-year precipitation mean for most months (Fig. 1, DWD 1999, 2000, 2001).

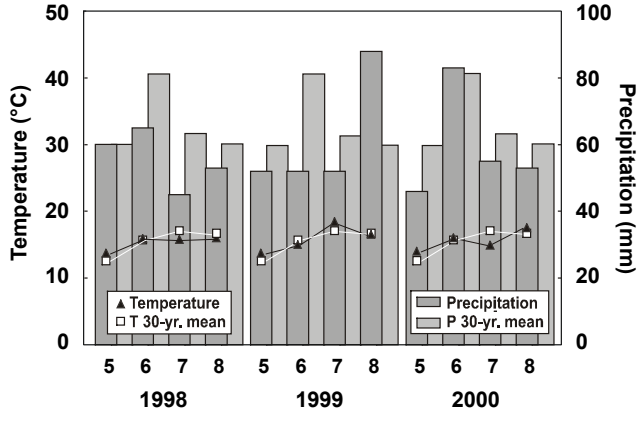

Fig. 1 Temperature and Precipitation at Göttingen during the three years of study (DWD 1999, 2000, 2001). Mean monthly temperature and precipitation are contrasted to the 1961-1990 long year means.

Plot design and treatments

Study plots were established on an 2 year old fallow densely covered with thistles in May 1998 (mean thistle density of 70 ± 28 thistles/m² at the first sampling date in May 1998). 75 plots of 2 x 2 m² were marked with wooden stakes in a block of 5 x 15 in 1998, with a spacing of 3 m between plots. Interspace rows were mowed once every year in July. 72 plots were randomly assigned to eleven treatments with six replicates each (control: 12 replicates). In the 11 treatments, repeated each year in 1998, 1999 and 2000, we applied spore suspensions of *P. punctiformis* and *P. destructiva*, each once and in repeated inoculations, singly and combined, and compared them with a fungicide treatment and a control (see Table 1 for details).

Urediniospores of *P. punctiformis* were collected at the end of May of each year from systemically infected thistles on a grassland patch south of Göttingen. This site was densely covered with thistles and had a high incidence of systemic infections. Infected thistles were transferred to the laboratory, slightly dried at room temperature and spores separated by shaking individual thistles in a plastic box. Spores adhering to the plastic surface could then be brushed off with a paint-brush. Urediniospore suspensions consisted of 400 mg spore material (fresh weight) mixed with 800 ml of water, adding a drop of detergent (TWEEN® 40, Polyoxythylene sorbitan monopalmitate, Merck-Schuchardt, Germany), resulting in a concentration of approximately 1.1 x 10^5 spores/ml. Spores within suspension had germinated readily in germination experiments on water agar in the laboratory. Each plot was treated with 67 ml of spore suspension (equalling 71 x 10^5 spores/m²) that was applied with an atomiser from all sides of each plot.

P. destructiva was isolated from chlorotic thistles following Guske (1995). The infected shoots were collected on a fallow at the Reinshof site. Spores of *P. destructiva* were obtained

Table 1 The eleven treatments applied to each of six thistle plots in each of the three study years (control: 12 plots).

Treatment No.	*P. punctiformis*			*P. destructiva*			Fungicide	Control
	June	July	August	June	July	August	June/July	
1	x							
2		x						
3			x					
4	x	x	x					
5				x				
6					x			
7						x		
8				x	x	x		
9	x			x				
10							x	
11								x

by homogenising mycelium cultivations of *P. destructiva* on malt-agar in sterile water. The homogenate was vacuum-filtered, centrifuged and then resuspended. Spore concentration was counted in a Thoma counting chamber and diluted with sterilised water to a concentration of 1.5 x 10^6 spores/ml. Conidiospore suspensions of *P. destructiva* with a concentration of 1.5 x 10^6 spores/ml were used. One drop of detergent (TWEEN® 40) was added to each litre of suspension. Each experimental plot was treated with 67 ml of spore suspension applied with an atomiser, equivalent to 25 x 10^6 spores/m². Spore suspensions were always applied late in the evening at increased humidity to facilitate infection.

The fungicide Opus Top® (BASF, 84g/l epoxiconazol and 250g/l fenpropimorph) was applied twice during the growing season to control *P. punctiformis*, at concentrations recommended by the producer (1500 ml Opus Top per ha, equalling 3,6 ml Opus Top diluted with 1000 ml of water per six plots). Opus Top® is a systemic fungicide effective against fungi in cereals, especially of the genus *Puccinia*. Spraying dates were 6 June and 30 June 1998, 8 June and 6 July 1999, and the 14 June 2000.

Measurements

Plots were monitored monthly during the growing season of each year (1998: 11/5, 9/6, 7/7, 4/8, 1/9; 1999: 4/5, 7/6, 5/7, 2/8, 24/8; 2000: 3/5, 8/6, 1/7, 2/8). The number of thistles that were not systemically infected with *P. punctiformis* or infected with *P. destructiva* ("healthy"), and the number of thistles systemically infected by *P. punctiformis* or *P. destructiva* was counted. The height of the ten tallest "healthy" thistles was measured. The number of flower buds of the ten tallest "healthy" thistles was counted in August. Vegetation relevées were made monthly on the plots and the cover of thistles and other species was estimated in percent following the Londo-scale (Londo 1975).

In half of the plots (treatments 1-4, 10 and 11, see Table 1), the effects of inoculations with *P. punctiformis* were measured by sampling leaves of thistles. Three leaves from 10 randomly chosen plants (1998), respectively two leaves from 8 randomly chosen plants (1999, 2000) per plot were sampled and the number of fungus sori was counted under a stereo microscope. Leaf area was estimated (Kluth et al. 2002c) and the number of sori was related to leaf area.

Statistical analyses

Data were tested for normal distribution and log-transformed where applicable to meet the assumptions of normality. To test the impact of treatments, simple ANOVA and nested ANOVA with repeated measures (monthly measurements nested in yearly repetitions, or

yearly repetitions only) were performed with STATISTICA (StatSoft, Inc. 2000). Differences among means were compared with post-hoc Tukey tests for unequal *n*. As the initial total numbers of thistles per plot were slightly different among the treatments at the initial counts in May 1998, we calculated relative thistle number per plot by relating counts to the initial total number of thistles of every plot, defined as 100%. Relative growth rate was determined as RGR=(ln h_2-ln h_1)/Δt (Hunt 1989), with h_1 and h_2 being the mean height of thistles at the time of measurement in June and August of the same year, and Δt the number of days between measurements. Means ± standard errors are given in both text and graphs.

Results

Although thistle plots were placed in a visually homogenous, densely thistle-covered fallow at the beginning of the study in 1998, the abundance and distribution of thistles changed extraordinarily during the three-year treatment period. First year counting of thistles in May resulted in an overall mean density of 56.5 ± 2.4 thistles/m², with maximum densities of up to 105 thistles/m². Towards 1999, density was gradually reduced, in May 1999 the overall mean being 29.6 ± 1.5 thistles/m². This was followed by a rapid decline in 2000. By May 2000, the overall mean was 4.3 ± 0.5 thistles/m², with 20% of the plots being free of all thistles. The decline in thistle number was reflected in thistle cover, which also decreased dramatically over the three year period (Fig. 2A). At the same time, the cover of co-occurring species, predominantly *Epilobium* species and grasses, increased, which was reflected in a significant decline of the thistle to grass cover ratio (Fig. 2B, Table 2). In 1998, thistle cover was 30 fold higher than, for example, the cover of *Elymus repens*. In 2000, *E. repens* cover was almost twice that of *C. arvense*.

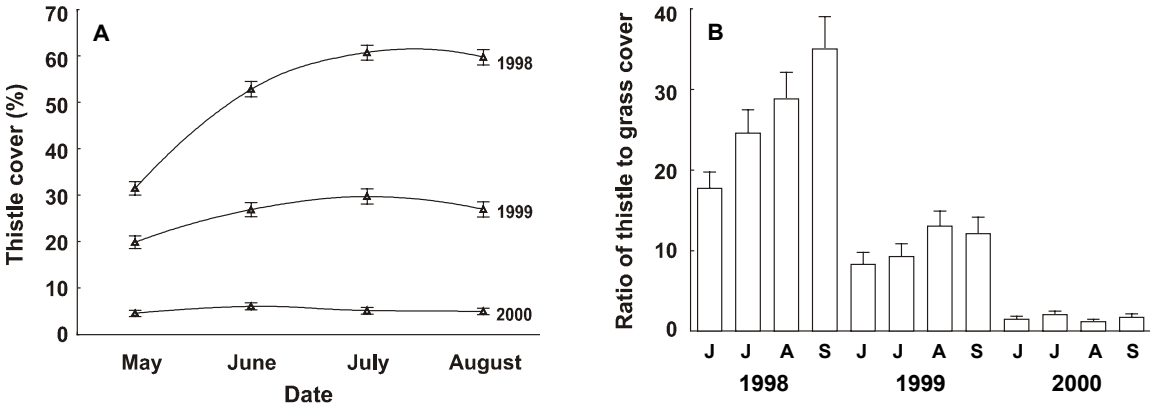

Fig. 2 Plant cover changes over the three year study period. **A)** Thistle cover. Lines through means are for descriptive reasons only. Nested ANOVA with repeated measures. Year: $F_{2,11}$ =634; P<0.001; Month: $F_{9,11}$ =133; P<0.001. **B)** Ratio of thistle to grass cover during the three year study period. Nested ANOVA with repeated measures. Year: $F_{2,64}$ =23.1; P<0.001; Month: $F_{9,288}$ =6.37; P<0.001.

Table 2 August cover of the eight most common species on the study plots during the three years of treatment. Means and standard errors are given.

Nr	Species	1998	1999	2000
1	*Cirsium arvense* (L.) Scop.	60.2 ± 1.8	26.9 ± 1.7	4.9 ± 0.7
2	*Epilobium* spp.[1]	9.1 ± 1.0	38.5 ± 2.1	47.2 ± 2.7
3	*Urtica dioica* L.	4.5 ± 0.8	7.7 ± 1.5	7.5 ± 1.3
4	*Galium aparine* L.	3.2 ± 0.4	0.0 ± 0.0	1.0 ± 0.2
5	*Elymus repens* (L.) Desv.	3.1 ± 1.0	3.7 ± 1.2	9.1 ± 2.5
6	*Rumex obtusifolius* L.	0.8 ± 0.3	1.4 ± 0.5	0.6 ± 0.2
7	*Poa trivialis* L.	0.3 ± 0.1	1.1 ± 0.3	3.1 ± 0.7
8	*Arrhenaterum elatius* (L.) J. et C. Presl	0.1 ± 0.0	1.7 ± 0.7	5.7 ± 1.9

[1] *E. tetragonum. E. lamy.* and *E. hirsutum* combined

No significant effect of triple inoculations with either *P. punctiformis* or *P. destructiva*, compared to a single inoculation at each date, could be found. Likewise, inoculation dates of single fungus applications did not make a difference (tested in a separate ANOVA with the four *P. punctiformis* inoculations, respectively the four *P. destructiva* inoculations only). We therefore decided to pool the data, resulting in five treatments for further statistical analysis: application of *P. punctiformis*, application of *P. destructiva*, combined application of both fungi, a control, and the fungicide treatment.

Thistle abundance

The relative number of all thistles per plot was significantly reduced by factors year and month, tested in an ANOVA with repeated measures (Table 3, illustration of August data Fig. 3A). The interaction of treatment with year, and with month, was significant. Second-year data, tested separately, indicated a marginally significant effect of treatments ($F_{4,67}$ =2.12; P=0.088) and post-hoc Tukey tests for unequal n revealed a reduced thistle abundance in the combined inoculation with *P. punctiformis* and *P. destructiva* compared to the fungicide treatment in August (Fig. 3A, $\alpha < 0.1$).

Table 3 The effect of treatments on the relative number of thistles (Fig 3A), on mean height of the ten tallest "healthy" thistles, and on sorus density of *P. punctiformis* (Fig. 6A). Nested ANOVA with repeated measures.

Effect	Relative thistle number (%)	Height of the ten tallest thistles (cm)	Sorus density of *P. punctiformis* (cm $^{-1}$)[1]
Treat	$F_{4,67}$ = 1.91	$F_{4,42}$ = 0.17	$F_{4,67}$ = 10-1***
Year	$F_{2,134}$ = 529***	$F_{2,84}$ = 511***	$F_{2,134}$ = 4.79*
Month	$F_{9,603}$ = 142***	$F_{9,378}$ = 1156***	$F_{9,603}$ = 0.78
T*Y	$F_{8,134}$ = 2.20*	$F_{8,84}$ = 2.00 (*)	$F_{8,134}$ = 0.12
T*M	$F_{46,603}$ = 1.83**	$F_{46,378}$ = 5.41***	$F_{46,603}$ = 3.02**

[1] *P. punctiformis* treatments, fungicide treatment and control in 1998 and 1999 only.
(*) P<0.1; * P<0.05; ** P<0.01; *** P<0.001; otherwise P>0.05.

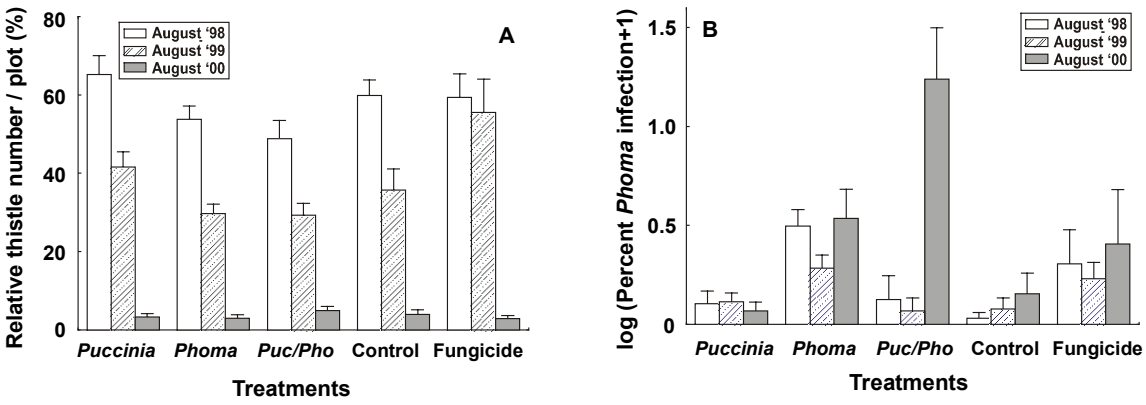

Fig. 3 Influence of treatments on thistle abundance over three consecutive years. **A)** Relative thistle number per plot in August. Nested ANOVA with repeated measures, for statistics see Table 3. Graphs show means and standard errors. **B)** Percent thistles of total number of thistles infected with *P. destructiva* in August of the three consecutive years. ANOVA with repeated measures for log-transformed data. Treatment: $F_{4,67}$ = 8.04; P<0.001; Year: $F_{2,134}$ =11.4; P<0.001; Interactions: $F_{2,134}$ =4.69; P<0.001. Graphs show means and standard errors.

Treatments did not affect the abundance of thistles systemically infected with *P. punctiformis*. Percentages of systemically infected thistles were extremely high in 1998 with 29.5% ± 1.9% in May 1998, but declined with the overall decline of thistle number (7.5% ± 1.7% in May 2000). This was reflected in the significance of year as one repetitive factor in nested ANOVA with repeated measures (Year: $F_{2,134}$ =96.4; P<0.001).

P. destructiva infection, resulting in obvious plant chloroses, occurred in only a few thistles with a mean percentage of 1.8% ± 0.4% infected thistles per plot in August 1998, but this percentage increased to 7.2% ± 1.8% infected thistles per plot in 2000. Relative *P. destructiva*-infection was influenced by both treatment and year, with a significant interaction effect of both factors (August data, Fig. 3B). In 1998, *P. destructiva* inoculations significantly increased the percent infected plants per plot compared to controls and *P. punctiformis* inoculations (ANOVA with 1998 data only. $F_{1,71}$ =5.96; P<0.001; post-hoc Tukey tests for unequal n). In 2000, the synergistic effect of combined *P. punctiformis* and *P. destructiva* inoculations significantly increased the percent infection with *P. destructiva* compared to controls and *P. punctiformis* inoculations (ANOVA with 2000 data only. $F_{1,71}$ =9.50; P<0.001; post-hoc Tukey tests for unequal n).

Vegetative performance of "healthy" thistles

The mean height of the ten tallest "healthy" thistles was not influenced by the different treatments, but by year and a significant time x treatment interaction (Table 3). Whereas treatments did not influence thistle height in 1998, thistles in single *P. destructiva* and combined *P. punctiformis* and *P. destructiva* inoculations were taller, and those of single

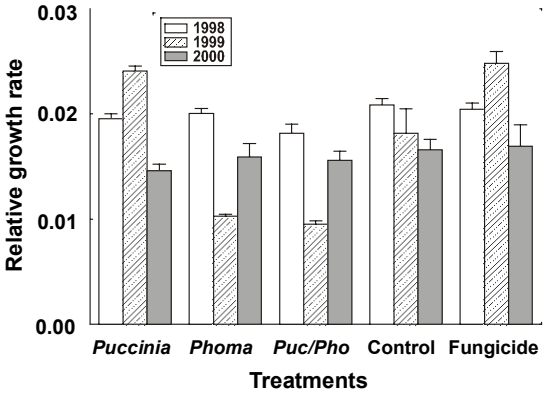

Fig. 4 Relative growth rate of ten tallest "healthy" thistles. ANOVA with repeated measures. Treatment: $F_{4,42}$ =12.4; P<0.001; Year: $F_{2,84}$ =15.7; P<0.001; Interactions: $F_{2,84}$ =19.0; P<0.001. Graphs show means and standard errors.

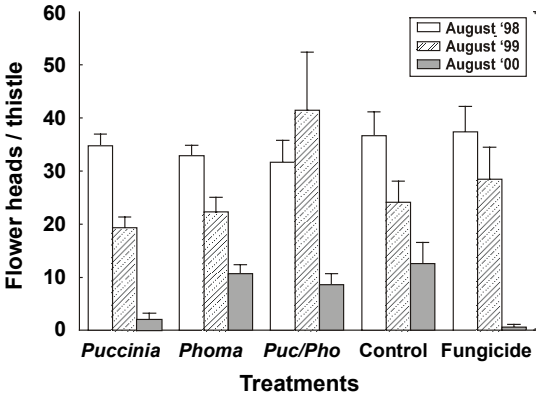

Fig. 5 Effect of treatments on the number of flower heads per "healthy" thistle in August 1998, 1999 and 2000. ANOVA with repeated measures. Treatment: $F_{4,45}$ =2.85; P=0.034; Year: $F_{2,90}$ =86.1; P<0.001; Interactions: $F_{8,90}$ =3.17; P=0.003. Graphs show means and standard errors.

P. punctiformis and fungicide treatments were smaller than controls in May 1999. Due to the significantly reduced growth rate of thistles in both singly *P. destructiva*-treated plots as well as combination treatment plots, and increased growth rates in *P. punctiformis* and fungicide treated plots (ANOVA with 1999 data. $F_{4,67}$ =56.1; P<0.001, Fig. 4), this difference was balanced by the next months' measurement and thistle height was no longer different among the treatments.

Sexual reproduction

The number of flower heads per "healthy" thistle was significantly reduced in 1999 compared to 1998 (P<0.001) and marginally influenced by treatments. First year treatments did not influence flower head production significantly, whereas second and third year treatments increased the number of flower heads in combined fungus treatments compared to single *P. punctiformis* inoculations (ANOVA for 1998 data: $F_{4,67}$=3,19; P=0.019), and reduced the number of flower heads in *P. punctiformis* inoculations compared to controls, respectively (ANOVA for 1999 data: $F_{4,45}$=6.05; P<0.001; Fig. 5).

Sorus density of P. punctiformis

Sorus density of *P. punctiformis* was significantly influenced by both treatment and year (Fig. 6A, Table 3). Fungicide application significantly reduced sorus density compared to controls and *P. punctiformis* inoculations at all dates, whereas the sorus density of controls was not different from the sorus density of *P. punctiformis*-treated plots. Nevertheless, the density of sori in July 1998 correlated positively with the number of systemically infected thistles in the following spring (Fig. 6B), whereas there was no correlation between numbers of

systemically infected thistles in 1998 with numbers of systemically infected thistles in 1999.

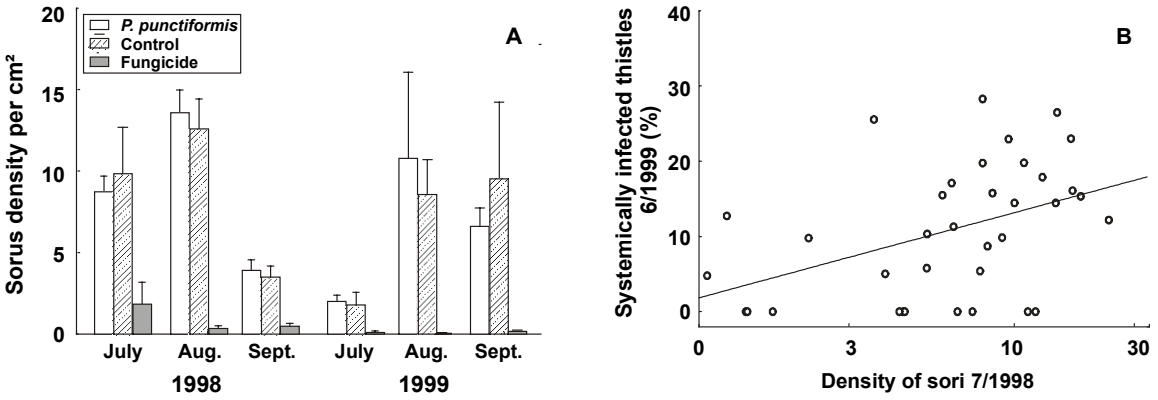

Fig. 6 Sorus density of *P. punctiformis* and systemically infected thistles **A)** Influence of treatments on *P. punctiformis* sorus density over three consecutive years. Nested ANOVA with repeated measures, for statistics see Table 3. Graphs show means and standard errors. **B)** Influence of the log mean density of sori, measured on leaves of *C. arvense* in July 1998, on the percentage of systemically infected thistles in June of the following year. Pearson product-moment correlation. N=36; r = -0.43; r^2 =0.19; P=0.008; y=1.84+10.8 x.

This was different in 1999 and 2000, when the number of systemically infected thistles in May 2000 depended on infected thistle number of the previous May (N=72; r=0.59; r^2=0.35; t=6.14; P<0.001; y=0.07 + 0.19x).

Discussion

EU policy restricts the use of chemical herbicides on set-aside land. Successful application of mycoherbicides to effectively control weeds would thus be an ideal treatment scheme for fallow fields. This three-year experiment was performed at natural site conditions to test the effect of two fungi on thistle performance. The study site was initially chosen because of its high thistle cover of almost 60%, nevertheless there was a rapid breakdown of thistle populations within the three study years, together with an increased densitiy of co-occurring vegetation, mainly *Epilobium* species and grasses. Schmidt (1981) studied natural and experimental succession on fallow land and found an increase of up to 5-15% thistle cover in the third and fourth year of succession, followed by a decline. Denys & Tscharntke (2002) also observed thistle densities to decline in field margin strips with increasing age, whereas *E. repens* densities increased. *E. repens* was also the predominant grass species in our study plots and competition effects are likely to cause the thistle decline. The negative effect of competition on thistles is known from several other studies (Holm et al. 1977, reviewed in Donald 1990). Holm et al. (1977) report that thistle seedlings are extremely sensitive to competition, while vegetatively propagated shoots easily penetrate ground cover, but suffer from retarded growth if grass provides strong competition. The naturally occurring, strong

degree of localised infection with *P. punctiformis* might have contributed to thistles being weak competitors.

P. punctiformis *inoculation*

No effect of *P. punctiformis* inoculations was found on the abundance of systemically *P. punctiformis*-infected thistles. This might be explained by the high amount of inoculum present on all plots due to unusually high percentages (near 30%) of systemically infected thistles in the first year, compared to Frantzen (1994a) and Kluth et al. (1997) report systemic rust infection to occur at percentages of below 5%. Because the abundance of systemically infected thistles in 1999 was not influenced by the systemically infected thistle abundance of the previous year, 1998, inoculum can be expected to have been evenly dispersed at high densities across the study plot. This was confirmed by sorus counts, revealing no effect of single or triple *P. punctiformis* inoculations at high spore densities on the degree of localised infection of thistles in treated plots compared to the degree of natural infection on control plots. Hence, an additional impact of artificial inoculation might be difficult to achieve in field situations where densities of systemically infected thistles are already high. Inoculation of healthy plants nevertheless has been shown to increase the density of sori on thistle leaves in pot trials and, as a consequence, the number of systemically infected thistles in a subsequent study year (Kluth et al. 2002c). This same relationship between localised infection and systemic infection in the following year was confirmed here at field conditions.

Three year inoculations with *P. punctiformis* did not result in differences in maximum thistle height, whereas we observed a strong negative effect of *P. punctiformis* inoculations on flower formation. This is contrary to former studies which describe localised *P. punctiformis* infection to have little effect on thistle growth and merely emphasise the effects of systemic infection (Bailiss & Wilson 1967).

P. destructiva *inoculation*

The percentage of thistles showing chloroses as a clear sign of *P. destructiva* infection was increased in *P. destructiva*-inoculations, especially in the first year of treatments. has been shown to result in a reduced flower bud formation due to infection (Kruess, unpublished data). In contrast, flowering of "healthy" thistles in the same plots was not influenced by the *P. destructiva*-inoculation.

Combined inoculations

Combined fungus inoculation with both *P. punctiformis* and *P. destructiva* resulted in a synergistic negative effect on thistle abundance in the second year of study. Stress by

P. punctiformis also increased the percentage of successful infection with *P. destructiva*. Boyle & Schulz (1994) argued that double infections with a biotroph (*P. punctiformis*) and a perthotroph (*P. destructiva*) could be very effective because the biotroph provides a way of entry for the perthotroph by rupturing the plant surface at sporulation. Similar positive results of double inoculations were observed by Hallett & Ayres (1992) and Hallett et al. (1990a, b). They found secondary infections with the fungus *Botrytis cinerea* to be facilitated and mortality increased in groundsel *Senecio vulgaris* previously infected with *Puccinia lagenophorae*. Despite the increased initial size of thistles, that were inoculated with both fungi, in May 1998, as well as the increased flower head production, both height and flower head number were reduced to values similar to control values by May 2000. This indicates that the application of two fungi, both inducing stress on thistles, might be able to reduce thistle abundance successfully compared to one fungus alone (Boyle & Schulz 1994, Guske 1995).

Fungicide

Although the number of systemically infected thistles in fungicide-treated plots of 1999 and 2000 could be expected to be lower than in plots without fungicide treatment, this was not the case. During the study, we observed that systemically infected shoots developing in fungicide plots lived several weeks longer than those in other plots. A possible explanation could be that fungicide also reduced the impact of *P. punctiformis* within these plants.

Height of thistles was not different among the different treatments, although relative growth rate in fungicide treatments tended to be increased. Thistles in fungicide plots were only slightly taller, but looked more vigorous than thistles in treated plots (Kluth, personal observation). This indicates that there might be a noticeable negative effect of *P. punctiformis* on performance of thistles, contributing to the dramatic reduction of thistle density during the three years of the study. Thistle number and height might not be ideal parameters to measure the impact of pathogens on thistle fitness. However, estimating performance of thistles by biomass measurements would have influenced underground thistle root systems and storage (Kluth et al. 2002c) and would also have severely disturbed the plots. A final biomass determination after the end of the experiment was intended, but no longer useful, since 30% of the plots were free of thistles, independent of treatment.

In conclusion, we showed long-term effects of two pathogenic fungi, both alone and combined, adding to competition with grasses which seriously reduced thistle densities in the third year of study. Biological control with pathogens may be a means of thistle control, especially in ecological conservation sites and organic farming, whereas the treatment of

crops with fungicides releases weeds from their natural pathogen enemies, which, in turn, might aggravate weed calamities.

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Insects as vectors of plant pathogens: mutualistic and antagonistic interactions

5

Abstract Interactions between plants and their herbivores and pathogens are mostly analysed separately, thereby neglecting mutualistic or antagonistic interactions between these antagonists and possible joint effects on the host. We studied interactions between the weed *Cirsium arvense*, the rust fungus *Puccinia punctiformis* and three herbivorous insects, the aphids *Aphis fabae* ssp. *cirsiiacanthoidis* and *Uroleucon cirsii*, and the beetle *Cassida rubiginosa*. All three insect species mechanically transported spore material and significantly increased rates of *P. punctiformis* infection in healthy thistles. The interaction between *C. rubiginosa* and the fungus was antagonistic. Although *C. rubiginosa* transferred spores, biomass of adults was significantly reduced, development of adults tended to be prolonged and mortality increased when feeding on plants infected with *P. punctiformis*. In contrast, the relationship between the aphid *U. cirsii* and *P. punctiformis* was mutualistic: *U. cirsii* profited by fungal infection and formed significantly larger colonies on fungus-infected plants. Although the differences in insect performance suggest that aphids may be better vectors than the beetle, infection rates were similar. This is the first study to demonstrate that the relationship between herbivores increasing the dispersal of a pathogen and the pathogen itself can be both mutualistic or antagonistic, depending on the species.

Keywords *Cirsium arvense*, *Puccinia punctiformis*, Plant-fungus-insect interactions, Insect performance, Herbivory

Introduction

Increasing evidence suggests that microorganisms within plants mediate trophic interactions between herbivores and their host plants (Clay 1988, Barbosa 1991, Hammon and Faeth 1992, Hatcher and Ayres 1997, Moran 1998). Many studies have documented interactions between endophytic fungi, their host plants and herbivores (e.g. Webber 1981, Breen 1993, Clement *et al*. 1996, Clay 1996, Saikkonen et al. 1996, Faeth and Hammon 1997, Williamson and Potter 1997, Wilson and Carroll 1997, Raps and Vidal 1998, Tibbets and Faeth 1999). These interactions between plants and endophytic fungi have been characterised as mutualistic and detrimental to herbivores (Cheplick and Clay 1988, Clay 1988, Raps and Vidal 1998). Recent studies question the general mutualistic nature of this relationship (Hammon and Faeth 1992, Clay et al. 1993, Lopez et al. 1995) and find interactions between hosts, endophytes and

herbivores likely to be highly variable (Breen 1993, Saikkonen et al. 1996, Faeth and Hammon 1997, Saikkonen et al. 1999, Tibbets and Faeth 1999).

Compared to studies on endophytic fungi, studies on the relationship between herbivores and plant pathogenic fungi on the same host plant are rare (but see Zebitz 1990, Hatcher et al. 1994, Hatcher 1995, Paine et al. 1997, Moran 1998, Friedli and Bacher 2001b, Hatcher and Paul 2001, Kluth et al. 2001, Kruess 2002). In few fungal diseases the role of insects in the transmission of pathogens has been thoroughly investigated (Agrios 1980, Webber and Gibbs 1989), but the possible mutualistic or antagonistic interactions among herbivores and pathogens have mostly been neglected (but see Klepzik et al. 2001). Furthermore, understanding transmission of fungi by insects is important for management of crops and weeds.

In our study we focused on tripartite interactions between herbivorous insects and the rust fungus *Puccinia punctiformis* (Str.) Röhl (Uredinales) on their host plant *Cirsium arvense* (L.) Scop. Previous field studies had shown that aphid species specialized on thistles preferred those thistles that were systemically infected with the pathogen *P. punctiformis*, whereas the leaf beetle *Cassida rubiginosa* Müll. (Coleoptera, Chrysomelidae) preferred healthy leaves (Kluth et al. 2001). For our experiments we chose the aphids *Uroleucon cirsii* L. and *Aphis fabae* ssp. *cirsiiacanthoidis* Scop. (Hemiptera: Aphidae) and the beetle *Cassida rubiginosa*. We hypothesised that these herbivores promote host plant infection and benefit from visiting infected plants. Based on our previous studies of plant preferences in the field (Kluth et al. 2001), the transport of spore material by the aphid *Uroleucon cirsii* and other specialized aphids seemed likely.

To our knowledge this is the first study to show experimentally that insects act as vectors of urediniospores of the autoecious rust fungus, resulting in mutualistic or antagonistic pathogen-insect interactions.

Materials and methods

The study species

The Creeping thistle *Cirsium arvense* is a noxious weed that is difficult to control due to a deep-growing root system (Donald 1994). The difficulty of control and side effects of herbicide use have prompted interest in biological control of thistles by natural antagonists. The aphid *Uroleucon cirsii* is an aphid specialized on *Cirsium* species as host plants. From May onwards, colonies occur on the upper parts of stems and on the upper leaves (Heie 1994). The primary host plants of the black thistle aphid, *A. fabae* ssp. *cirsiiacanthoidis* are *Euonymus*, *Viburnum* and *Philadelphus* species, and thistles are the secondary host. Colonies

on thistles are found on stems and leaves (Heie 1994). The oligophagous beetle *Cassida rubiginosa* is specialized on Cardueae and common on thistles. Adults hibernate and lay eggs in late spring, larvae then feed on leaves of thistles from June to August (Freude et al. 1983). The autoecious rust fungus *P. punctiformis* produces urediniospores and teliospores, but no aecidiospores. Urediniospores infect above ground plant parts and cause local infections on leaves with lesion damage. Sori develop locally on the leaves and release uredinio- and teliospores that further spread infection. Teliospores infect thistles via the root buds and form a systemic mycelium within the plant. Sori develop all over the leaves and shoots of thistles and also release uredinio- and teliospores. Compared to local infections of leaves, that influence thistle performance only marginally (Kluth, unpublished data), systemic infection strongly alters thistle life cycles: plants die early and seldom flourish (Buller 1950). Nevertheless, an increase in local infection rate naturally enhances the amount of teliospore inoculum and probably increases the rate of systemically infected thistles.

Experiments

We performed three experiments to study whether the selected herbivores enhance host plant infection and profit from infection or not. In the first vector experiment, we tested the insects' capability to promote infection via spore transport and determined the effect of infection on the reproduction of aphids. In the second vector experiment, we additionally inoculated thistles manually to evaluate the effect of the presence of herbivores and to enhance infection rates. Finally, in the *Cassida* performance experiment, we analysed how local and systemic fungus infection influences the development of *Cassida* larvae.

Thistles

Thistles were reared from root fragments. For the first vector experiment, horizontal roots were taken from a field densely covered with thistles in April 2000. We assured that the roots for each experiment originated from one clone by digging connected pieces. Horizontal roots of similar diameter were cut to 3 cm length, planted in standard soil in the glasshouse and, after two weeks, transplanted to 4 l pots. The similar size of root fragments reduces the probability of maternal effects. Pots were brought into the field in the beginning of May. Roots for the second vector experiment were taken in June, also from a fallow field, treated accordingly and brought into the field in July.

Vector experiment 1

The ability of insects to act as vectors was tested in a fully randomised design. We collected alate aphids of the species *Uroleucon cirsii* and *Aphis fabae* ssp. *cirsiiacanthoidis* and adults

of the tortoise beetle *Cassida rubiginosa* in the field in the beginning of June from non-infected thistles. Alate females of *U. cirsii* or of *A. fabae* ssp. *cirsiiacanthoidis* and adults of *C. rubiginosa* were placed in Petri dishes prepared with moist filter paper and 4-5 leaves of systemically infected *C. arvense*. Control aphids and beetles were placed on healthy leaves. After 24 hours, insects were carefully picked up with forceps and transferred onto the fourth stem leaf of potted healthy thistles, the forceps being carefully cleaned after each transfer. Either two aphids of one species or one beetle were placed on one leaf per plant. Insects on leaves were caged with gauze bags of 12 x 25 cm (mesh size 0.3 mm), control leaves were caged without insects. The set-up was replicated 16 times for *A. fabae* and 15 times for the two other herbivores for the infected and uninfected treatment, 15 control plants remained without herbivores (=107 potted thistles).

Vector experiment 2

In the second experiment with only *U. cirsii* and *C. rubiginosa* we proceeded as described above, but additionally inoculated leaves of healthy plants with an urediniospore suspension to increase the level of local infection. We thereby simulated a biological control treatment and were able to test the influence of the presence of herbivores on infection rates. The suspension consisted of 50 mg urediniospores diluted in 100 ml water plus one drop of detergent (TWEEN® 40, Polyoxythylene sorbitan monopalmitate, Merck-Schuchardt, Germany). One treatment comprised either two aphids or one beetle together with the fungus, the other was a rust-inoculated control without insects. There were 10 replicates for each of the eight treatments in this experiment. Due to unfavourable weather conditions leaves of 24 plants were torn off before harvest and were therefore not considered in the analysis.

In both experiments, aphids were left on the plants for 20 days, *Cassida* beetles were taken off after two days and leaves were caged again. After 20 days, leaves were collected, the number of urediniosori on each leaf was counted and the leaf size determined. The development of aphid colonies was recorded by counting the number of aphids per caged leaf.

Cassida *performance experiment*

We compared the performance of the tortoise beetle *Cassida rubiginosa* in relation to rust fungus infection of its host plant *C. arvense*. We studied whether the rust influences the development and mortality of larvae of *C. rubiginosa*, depending on local or systemic rust infection of thistles, compared to development and mortality on healthy thistles.

One newly hatched larva was placed in a petri dish and, in three different set-ups, fed with leaves of healthy, locally infected or systemically infected thistles. There were 15

replicates per set-up (n=45). Fresh leaves were given every two days and larval growth, development stage and mass increase were recorded. Every second day, larval size was measured under a dissecting microscope and larval mass determined.

Statistical analysis

Statistical analyses were performed with STATISTICA for Windows (Statsoft Inc. 2000). The effects of the transmission of *P. punctiformis* spores to healthy plants by insect vectors were compared in an analysis of variance. As transmission data did not meet the assumption of normal distribution for parametric ANOVA in the first vector experiment, we applied non-parametric tests. Comparisons within species (Fig. 1a) were performed with Mann-Whitney U-tests, among species with Kruskal-Wallis-ANOVA. Data on colony sizes was normally distributed and compared with ANOVA. Data of the second vector experiment were log (x+1) transformed to meet assumptions of normality and compared with ANOVA. Development of *Cassida* larvae was fitted with logistic regressions for descriptive reasons. Mortality of *C. rubiginosa* feeding on healthy, locally or systemically infected leaves was tested with Pearsons chi-square test for goodness of fit. Medians of time for *C. rubiginosa* larvae to develop from egg-hatch to adults and final mass of adults were compared with non-parametric Kruskal-Wallis-ANOVA. Data of the *Cassida* experiment were corrected with the sequential Bonferroni-method (Sokal and Rohlf 1995). Graphs show arithmetic means and standard errors.

Results

Vector experiments and aphid reproduction

Thistles with insects having fed on fungus-infected leaves developed significantly more urediniosori than thistles with insects that had contact with only non-infected leaves (Fig. 1A). Median numbers of urediniosori per cm² of leaf area were zero for the treatments with herbivores from healthy leaves and for the controls without herbivores. Medians of treatments with herbivores from infected leaves were significantly higher with 0.29 sori/cm² for *A. fabae*, 0.50 sori/cm² for *U. cirsii* and 0.41 sori/cm² for *C. rubiginosa*, respectively. These three insect species were therefore shown to transport spores from infected to healthy leaves, as the infection rate of control leaves was very low and not significantly different from that of leaves with insects from healthy plants. Observations of herbivores from infected leaves under the dissecting microscope showed spore material that was attached to the legs and body of the herbivores. Aphids collected from infected thistles in the field also had spores attached to their body.

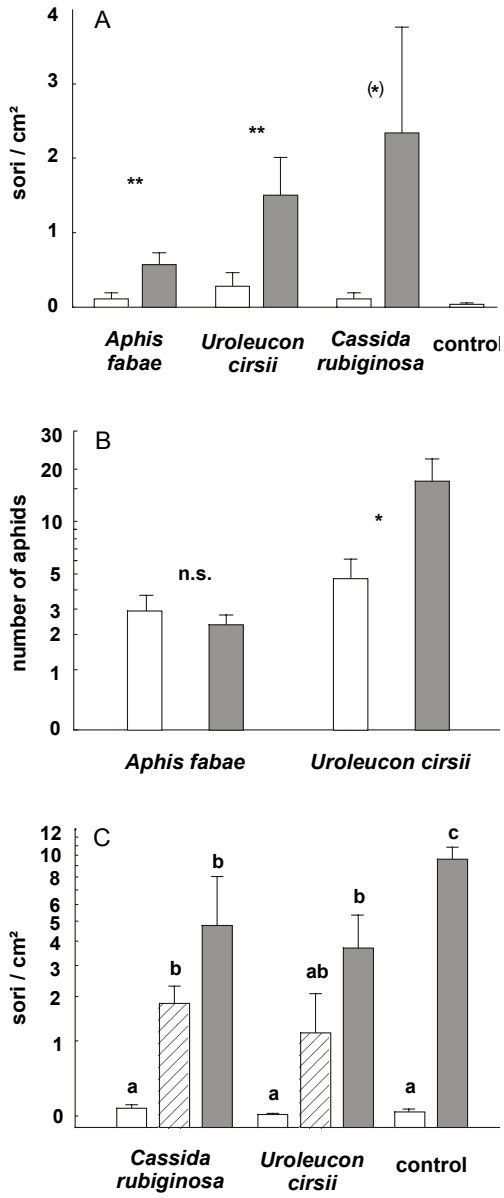

Fig. 1 Vector experiments and aphid reproduction.
A) Numbers of sori on thistle leaves with herbivores that came from healthy (white bars) or infected leaves (shaded bars). Mann-Whitney-U-test. *A. fabae*: U=52; n=32; P=0.003; *U. cirsii*: U=42; n=30; P=0.003; *C. rubiginosa*: U=68; n=30; P=0.067. Comparison of control with treatments with *Aphis fabae* ssp. *cirsiiacanthoidis*, *Uroleucon cirsii* and *Cassida rubiginosa* taken from healthy leaves. Kruskal-Wallis-ANOVA, H=2.08, n=61, P>0.3.

B) Size of aphid colonies on thistles after 20 days on healthy (white bars) or infected leaves (shaded bars; from the experiment shown in Fig. 1A). One-way ANOVA. *A. fabae*: $F_{1,31}$=0.001; n=32; P>0.3; *U. cirsii*: $F_{1,29}$=5.70; n=30; P=0.024.

C) Number of urediniosori on thistle leaves with herbivores that come from healthy (white bars) or infected leaves (hatched bars) or on manually inoculated leaves (shaded bars). One-way ANOVA. *U. cirsii*: $F_{1,23}$=7.52; n=24; P=0.003; *C. rubiginosa*: $F_{1,16}$=4.74; n=17; P=0.027; healthy control versus inoculated control: $F_{1,14}$=414; n=15; P<0.001. Means and standard errors are given. ** P<0.01; (*) P<0.1; * P<0.05; ns: not significant. Different letters denote statistically significant differences.

Aphids developed colonies during this 20 day-experiment (Fig. 1B). The number of *Aphis fabae* individuals was low, with means of 2.9 and 2.4 individuals on healthy thistles and thistles with aphid-induced infections, respectively. *Uroleucon* individuals also occurred in low numbers on healthy leaves (mean 4.7 individuals), but size of colonies was significantly larger on leaves that they had infected due to their spore transport (mean 16.5 individuals, Fig. 1B).

In the second experiment we also manually inoculated plants to test the effect of the presence of herbivores on infection at enhanced infection rates as proposed for biological control treatments (Fig. 1C). Results were similar to those already shown (Fig. 1A): both *C. rubiginosa* and *U. cirsii* acted as vectors and transported spores from infected to healthy plants,

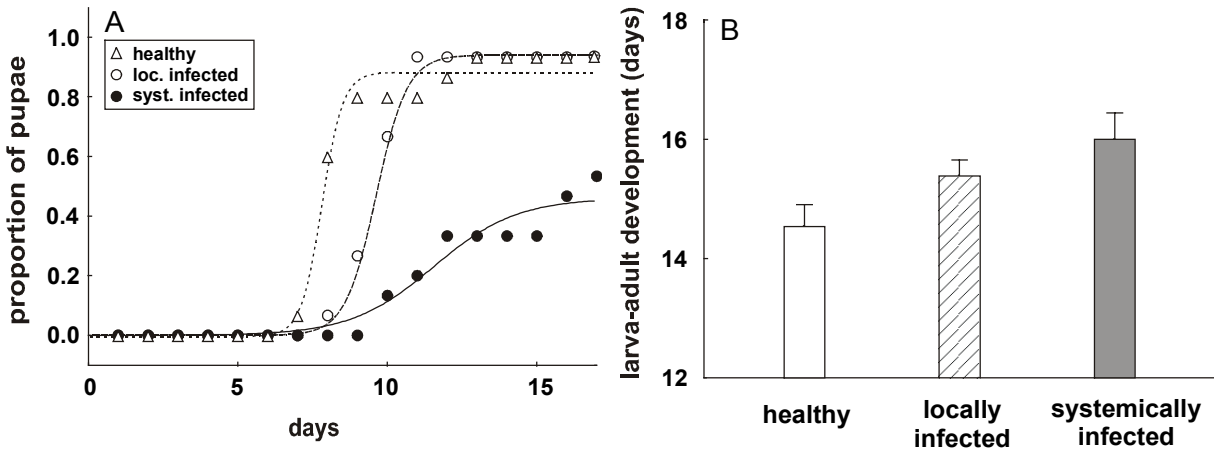

Fig. 2 Development of *C. rubiginosa* on the leaves of healthy, locally or systemically infected thistles. **A)** Proportion of pupae developing from larvae. Logistic regression model fitted for descriptive reasons. **B)** Development time from larvae (egg-hatch) to adults. Healthy: white bars, locally infected: hatched bars, and systemically infected: shaded bars. For statistics see Table 1. Means and standard errors are given.

thereby increasing the level of infection. The effect was not significant for *U. cirsii* at the $P<0.05$ level comparing only infected plants and controls, but highly significant for *C. rubiginosa*. Inoculation of leaves resulted in a significantly increased number of sori as well in insect treatments for both herbivores as in inoculated controls, compared to either treatment with insects coming from healthy plants or the control treatment, respectively (Fig. 1C). Whereas in both *C. rubiginosa* and *U. cirsii*, coming from healthy plants, the mean number of sori was 0.1 sori/cm², inoculation increased the numbers to 4.7 and 3.7 sori/cm², respectively. The degree of infection was significantly higher in the inoculated controls than in inoculated treatments with insects ($F=3.96$; $P=0.041$, Fig. 1C). Means of 4.7 or 3.7 sori/cm² with *C. rubiginosa* or *U. cirsii* contrasted with a mean of inoculated controls of 9.6 sori/cm².

Table 1 Summary of statistical results testing the effect of healthy (H), locally (L) and systemically (S) infected thistle leaves on mortality, development time (egg hatch to adult), adult mass and size of *C. rubiginosa*. Error probabilities passing a sequential Bonferroni correction with an experiment-wise error rate of $\alpha=0.05$ are printed in bold (error rate of $\alpha=0.1$ in brackets).

Parameter	Infection status	Mean ± SE	X^2 / H	P
Mortality[1] [%]	H	13.3 ± 1.1	X^2=6.71	**(0.035)**
	L	20.0 ± 1.1		
	S	53.3 ± 1.3		
Development time[2] [d]	H	14.5 ± 0.4	H=7.88	**(0.020)**
	L	15.4 ± 0.3		
	S	16.0 ± 0.4		
Adult mass[2] [g]	H	21.3 ± 1.0	H=8.97	**0.011**
	L	17.0 ± 0.8		
	S	19.1 ± 1.1		
Adult size[2] [cm]	H	1.17 ± 0.02	H= 2.30	>0.3
	L	1.14 ± 0.02		
	S	1.11 ± 0.03		

[1] Pearson chi-square test
[2] Kruskal-Wallis ANOVA

Cassida *performance experiment*

Cassida larvae developed faster on healthy than locally infected thistles (Fig. 2A, B, Table 1). 50% of the pupae that developed were found 7.8 days after egg-hatch on healthy, 9.5 days after egg-hatch on locally and 11.6 days after egg-hatch on systemically infected leaves. The proportion of larvae which succeeded to develop into pupae was 89% in healthy, 94% in locally infected thistles, and clearly reduced to only 46% by systemic infection of thistles (Fig. 2A). The overall mortality of larvae was also dependent on the infection of thistle leaves. Mortality was highest when larvae were feeding on systemically infected leaves whereas mortality was low on both healthy and locally infected leaves (Pearson chi square test, Table 1). Larva-adult-development was fastest on healthy leaves and became increasingly retarded with locally and systemically infected leaves (Fig. 2B, Table 1). Adult size was not significantly different among the treatments, but the final mass of *Cassida* adults was significantly reduced from 21.3 ± 1.0 mg, when feeding on healthy thistles, to 17.0 ± 0.8 mg, when feeding on locally infected thistles (Table 1).

Discussion

The mutualistic or antagonistic nature of interactions among herbivores and fungi on the same host plant is decidedly dependent on the species involved. Whereas several studies on endophytic fungi found negative effects of endophytes on herbivorous insects (Cheplick and Clay 1988, Clay 1988, Raps and Vidal 1998), in a recent review, Saikkonen et al. (1998) conclude that endophyte-host plant interactions are variable. Concepts of plant-endophyte interactions might be biased by focusing on mutualistic aspects of atypical cultivars and their generalist, rather than specialist, herbivores.

Our results confirm that interactions between a plant pathogen and herbivores on the same host plant are species specific: antagonistic in the leaf-beetle *C. rubiginosa* and mutualistic in the aphid *U. cirsii*, whereas there was neither an antagonistic nor mutualistic effect of pathogen infection on the development of *A. fabae* colonies. *U. cirsii* predominantly visits systemically infected compared to healthy thistles (Kluth et al. 2001). Here we could observe that their active role in thistle infection also positively influenced their reproductive fitness and increased colony sizes 3.5-fold compared to colony sizes on healthy thistles. Similar positive effects of rust infections on aphids have been found by Chang and Thrower (1981) and Zebitz (1990). Zebitz (1990) reported an infection *of Vicia faba* L. with broad bean rust to increase the susceptibility to infection of *Vicia faba* to *A. fabae*. Aphids were shown to favour and develop better on rust infected compared to healthy leaves. An increase in free amino-

acid concentrations in the phloem may be responsible for the beneficial effect of infection on sap-sucking insects (Bernays and Chapman 1994). Reproduction of *A. fabae* was not influenced by infection. Although Heie (1994) reports *A. fabae* to occur on both stems and leaves of thistles, we mostly observed *A. fabae* colonies on stems in the field. As in the vector experiments *A. fabae* were forced to feed on the leaves, the unsuitable habitat might explain the overall low rate of reproduction and the lack of differentiation between infected and uninfected hosts.

In contrast to positive effects of *P. punctiformis*-infection on *U. cirsii*, *C. rubiginosa* development was negatively influenced by host plant infection. Larvae developed more quickly on healthy thistles, whereas systemic infection of thistle leaves increased total development time and mortality. Final mass of adults was significantly higher in controls than in locally infected thistles. Hatcher et al. (1994), studying the effect of a rust disease on the development of a chrysomelid beetle, also found that all the beetle's life stages were inhibited when it was feeding on fungus-infected leaves. Kok et al. (1996) on the other hand stated no negative effect of rust-infected musk thistles on *C. rubiginosa*, but observed the beetles feeding mainly on rust pustule-free areas of the leaves. Kruess (2002) found similar negative effects of thistles infected with the fungus *Phoma destructiva* on larval development and survival of *C. rubiginosa*. This indicates that the negative impact of fungus infection on *C. rubiginosa* is independent of the pathogen species involved and rather a result of plant defence mechanisms than of pathogen toxins (Kruess 2002).

We found insects playing an important role in the distribution of spores of a plant-pathogenic fungus. All three herbivores studied significantly increased the rate of infection on healthy plants when they had previously fed on infected leaves. Both herbivores from the experiments and from the field were found to carry spores on their legs and body. A similar external transmission of fungal spores by insects is reported by several authors (reviewed in Agrios 1980, Bultman and White 1988, Webber and Gibbs 1989, Kok and Abad 1994, Paine et al. 1997, Morrison et al. 1998). Paine et al. (1997) reviewed the relationship between fungal pathogens and bark beetles vectors on conifers and described the relationship between both to be mutually beneficial. For the fungus, the vector offered a means of transport which was directed to suitable host species, the insects benefited from the provision of a suitable breeding habitat and food supply. As *U. cirsii* preferentially visits infected thistles in the field (Kluth et al. 2001) and aphid reproduction was increased on infected plants, we conclude that transport of spores by alate females results in a mutualistic benefit for both *U. cirsii* and the pathogen.

Compared to healthy plants, local infection already tended to retard *Cassida* development and significantly reduced adult weight. Although *Cassida* beetles avoided systemically infected plants in the field (Kluth, personal observation), they were frequently found on locally infected plants. The adaptive value of this behaviour seems unclear, but might be explained considering that adult females may have placed their eggs on formerly healthy plants, these becoming infected later on. This would not allow hatching larvae to escape infection. Also, by summer, about 95% of thistles usually becomes locally infected with *P. punctiformis* (Kluth, personal observation), demanding great skill and experience of beetles to discriminate the few remaining healthy from the infected plants. Even when feeding only on healthy parts of leaves, spores may attach to their body. The transport of spores can therefore be expected in the field for the beetle as well as for aphids, but resulting in an antagonistic effect for the beetle. The differences in insect performance suggest that aphids could be expected to be better vectors than *C. rubiginosa*, because they preferentially visit systemically infected thistles and profit from local infection. Early dead of systemically infected thistles in the field (Buller 1950, Van den Ende et al. 1987, Kluth, pers. obs.) will force them to move on to healthy or locally infected plants, thereby increasing the chance of further spore transmission. Zoochory of spores by specialized insects is selectively directed towards other host plants (Webber and Gibbs 1989, Paine et al. 1997). Compared to the general assumption that wind transport is the predominant mode of fungus dispersal, our results demonstrate the occurrence of insect transmission of spores that may also effectively spread the pathogen to isolated weed stands (Morrison et al. 1998).

In the second vector experiment we found competitive interactions between *P. punctiformis* and herbivores at high level degrees of inoculation. The presence of herbivores reduced the effect of manual inoculations on thistles compared to inoculated controls without insects. It is not likely that the reduction in urediniosori numbers is due to a reduction of leaf area, as *C. rubiginosa* beetles damaged less than 5% of leaf area during their two days on thistle leaves in our experiment. Rather, the reduction of infection rates may result from competition for nutrients of both herbivores and the pathogen at these high levels of infection or might be an effect of interactions in induced plant defence mechanisms, which are still not fully understood (Bostock 1999). In biological control, this might result in a trade-off between the positive effects of spore transmission by insects and the slight reduction of infection rates after artificial inoculation due to the presence of herbivores. Nevertheless, infection rates were still significantly increased by manual inoculations compared to untreated controls.

We conclude, that the importance of insects as vectors of fungal plant pathogens has often been underestimated (Agrios 1980). The use of *P. punctiformis* as a biocontrol agent will result in species-specific mutualistic and antagonistic interactions with herbivorous insects, but even negatively affected species such as *C. rubiginosa* may spread spores when switching from locally infected to healthy host plants. Further experiments will be needed to quantify the relative contribution of phytophagous insects and pathogens to the fitness of the weed *C. arvense*.

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Summary

There has been increasing interest in the biological control of weeds in the recent years, notably after successes in classical biological control have promoted the idea of biological control against native weeds with indigenous antagonist species. Although both herbivorous insects and pathogens have been tested for their suitability as biocontrol agents, few studies have considered possible interactions between these groups and their combined effects on their host plant. However, the knowledge of these interactions may be essential to estimate the effect of biocontrol measures.

Several antagonist species have been studied as possible biocontrol agents of creeping thistle (*Cirsium arvense*), which is considered worldwide as an important weed mainly because of its ability of vegetative reproduction. Interactions between these species have thereby mostly been neglected. Here, experiments with two pathogenic fungi, the rust fungus *Puccinia punctiformis* and the perthothroph *Phoma destructiva*, as well as several herbivorous insects have been performed to gain essential basic knowledge on biological thistle control.

The importance of thistle age and phenotype for susceptibility of *C. arvense* against *P. punctiformis* was studied both in common garden experiments and in field trials on a broader regional scale. Artificial inoculation of thistles with the rust *P. punctiformis* resulted in age-dependent differences in susceptibility in both field experiments and experiments with potted thistles. Young thistles were almost resistant, but resistance greatly decreased as plants matured. Comparing the susceptibility of thistle phenotypes from ten sites around the city of Göttingen, inoculation lead to a site-dependent degree of infection in both field experiments on a regional scale and in common-garden experiments, but the local pattern could not be used to predict the regional, between-site pattern.

The second experiment considered combined effects of pathogen application and cutting on the performance and nutrient storage of thistles within a two year study period. *P. punctiformis*-inoculation reduced above-ground biomass in the first year of treatments, while first year cutting reduced the number of thistle shoots, but stimulated compensatory growth with an increased growth rate and biomass. Only a second year cutting treatment significantly reduced both above-and below-ground biomass. The number of flower heads was strongly diminished by both cutting and combined treatments. The proportion of fertile flower heads nevertheless was only diminished in the combination treatment. Starch concentrations in thistle roots did not differ among treatments. Mowing reduced sugar concentrations, whereas

pathogen infection tended to increase them. Mowing also reduced below-ground carbon concentrations, but the carbon-nitrogen ratio was not influenced.

Third, we tested the effect of two fungi, *P. punctiformis* and *P. destructiva*, as possible biocontrol agents of thistles at natural fallow conditions. The three year field study revealed a strongly negative impact of competition on *C. arvense* by the co-occurring fallow vegetation, showing also in a significant reduction of thistle to grass cover ratio. Combined inoculations with both *P. punctiformis* and *P. destructiva* resulted in a synergistic reduction thistle abundance in the second year. The number of systemically *P. punctiformis* infected thistles was not influenced by treatments, but correlated significantly with the degree of local infection. Infection with *P. destructiva* in combined inoculations was enhanced in the third year. Localised infection with *P. punctiformis* on thistle leaves correlated positively with percentage systemic rust-infection in the following season. Further, localised infection significantly reduced flower bud production after three years.

In the fourth experiment we studied mutualistic and antagonistic interactions between specialist herbivores and the thistle rust *P. punctiformis*. All three insect species transported spore material and significantly increased the degree of rust infection in healthy thistles. The interaction between a leaf beetle and the rust was antagonistic. Although the beetle transferred spores, its performance was reduced after infection of thistles with the rust. In contrast, aphids profited by fungal infection and formed significantly larger colonies on fungus-infected plants.

A combination treatment with the pathogens *P. punctiformis* and *P. destructiva* at natural conditions which include the competition with other plant species, seems to be a promising approach in biological thistle control. Additional measures like repeated mechanical treatments were shown to be effective in reducing thistle performance. A possible synergistic effect of repeated cuttings and inoculations should improve weed control. Naturally occurring insects can be expected to promote infection as vectors of spores, although the effect of infection on insect performance is species specific. Further studies are needed to establish *P. punctiformis* and *P. destructiva* as mycoherbicides of creeping thistle and test their effect on thistles in crop fields.

Zusammenfassung

Das Interesse an einer biologischen Unkrautkontrolle mit Hilfe von natürlichen Gegenspielern hat in den letzten Jahren stark zugenommen. Erfolge im Bereich der Klassischen Biologischen Kontrolle haben die biologische Kontrolle einheimischer Unkräuter mit einheimischen Gegenspielern möglich erscheinen lassen. Geeignete Gegenspieler können z.B. Insekten oder pathogene Pilze sein. Erst in letzter Zeit untersuchte eine vergleichsweise geringe Anzahl von Studien die möglichen Interaktionen zwischen diesen Gegenspielern und ihre Auswirkungen auf die gemeinsame Wirtspflanze. Dabei könnten Wechselwirkungen zwischen den Organismen den Erfolg oder Mißerfolg einer Biologischen Kontrolle entscheidend beeinflussen.

Die biologische Kontrolle der Ackerkratzdistel (*Cirsium arvense*), die vor allem wegen ihrer erfolgreichen vegetativen Vermehrung weltweit als Unkraut gilt, wurde bereits in mehreren Studien mit verschiedenen Gegenspielern untersucht, mögliche Interaktionen zwischen diesen Gegenspielern bisher jedoch nur ungenügend betrachtet. Diese Arbeit untersuchte in vier Experimenten mit zwei Pathogenen, dem Rostpilz *Puccinia punctiformis* und dem perthotrophen Pilz *Phoma destructiva*, sowie verschiedenen herbivoren Insekten, grundlegende Zusammenhänge zwischen diesen Gegenspielern und ihre Auswirkungen auf die Distel.

Das erste Experiment erfaßte den Einfluß des Alters und des Phänotyps der Ackerkratzdistel auf den Erfolg einer Pilzinokulation mit *P. punctiformis*. Eine *P. punctiformis*-Inokulation von Disteln im Topfversuch zeigte ebenso wie die Inokulation von natürlichen Distelpopulationen eine altersabhängige Anfälligkeit der Distel: Junge Disteln waren nahezu resistent, verloren diese Resistenz aber mit zunehmendem Alter. Ein Vergleich von Phänotypen zehn verschiedener Herkünfte um Göttingen ergab herkunftsabhängig unterschiedlich ausgeprägte Anfälligkeiten. Dieselben Phänotypen im Topfversuch zeigten ebenfalls Resistenzunterschiede, diese konnten jedoch nicht zu einer Vorhersage der Anfälligkeit auf größerer räumlicher Skala im Gelände herangezogen werden.

Im zweiten Versuch wurde die Beeinträchtigung der Distel durch eine Rostpilz-Behandlung kombiniert mit einer mechanischen Behandlung über einen Zeitraum von zwei Jahren und der Effekt der Behandlung auf die Einlagerung von Speicherstoffen untersucht. Eine Inokulation verringerte die oberirdische Biomasse im ersten Behandlungsjahr, während einfache Mahd zwar die Anzahl der Disteltriebe reduzierte, zugleich aber infolge einer erhöhten Wachstumsrate Biomasseverluste kompensiert wurden. Erst eine Zweitbehandlung im Folgejahr reduzierte die ober- und unterirdische Biomasse entscheidend. Die Anzahl der Blütenköpfe wurde sowohl durch Mahd wie auch durch die kombinierte Behandlung vermin-

dert. Der Anteil fertiler Blütenköpfe jedoch nahm insbesondere bei kombinierter Behandlung deutlich ab. Die Analyse der Speicherstoffe ergab keine Beeinflussung des Stärkegehaltes durch eine der Behandlungen, jedoch eine Reduktion der Zucker-Konzentrationen durch Mahd, wogegen die Zucker-Konzentrationen durch eine Infektion erhöht waren. Mahd verringerte zudem die Konzentration an Kohlenstoffen, veränderte das C/N-Verhältnis aber nicht.

Der Einfluß einfacher und kombinierter Pilzbehandlungen mit *P. punctiformis* und *P. destructiva* über einen Zeitraum von drei Jahren mit jährlichen Behandlungen wurde im dritten Experiment untersucht. Konkurrenz durch die Begleitvegetation verringerte die Distel-Dekkung signifikant im Laufe der drei Untersuchungsjahre, ebenso wie das Verhältnis von Distel- zu Gras-Deckung. Im Gegensatz zu einer einzelnen Applikation beider Pilze kam es durch einen synergistischen Effekt bei Anwendung beider Pilze zu einer Verringerung der Häufigkeit der Disteln im zweiten Untersuchungsjahr. Die Anzahl systemisch Rost-infizierter Disteln war unabhängig von den Behandlungen, korrelierte jedoch signifikant mit dem Grad lokaler Infektionen. Die Kombinationsbehandlung erhöhte den Anteil *P. destructiva*-infizierter Disteln. Die Häufigkeit lokaler Rost-Läsionen auf den Blättern der Distel korrelierte positiv mit dem Anteil systemisch infizierter Disteln im Folgejahr. Darüberhinaus verminderte die Rostinfektion die Zahl der Blütenköpfe nach dreijähriger Behandlung.

Mutualistische und antagonistische Wechselwirkungen zwischen herbivoren Insekten und dem Rostpilz und eine Übertragung des Pilzes durch die Insekten konnten im letzten Experiment nachgewiesen werden. Alle drei untersuchten Insektenarten übertrugen Sporenmaterial des Rostes und riefen eine Infektion an vormals gesunden Pflanzen hervor. Die Beziehung zwischen einem Blattkäfer und dem Rost war antagonistischer Art, er war in seiner Entwicklung durch die von ihm induzierte Infektion der Distel beeinträchtigt. Im Gegensatz dazu profitierten Blattläuse von einer Infektion und bildeten größere Kolonien.

Eine Kombinationsbehandlung mit den Pathogenen *P. punctiformis* und *P. destructiva* scheint ein vielversprechender Ansatz zur biologischen Kontrolle der Ackerkratzdistel unter natürlichen Konkurrenzbedingungen zu sein. Durch mehrfache mechanische Behandlung ließ sich die Fitness der Disteln ebenfalls beeinträchtigen, wobei ein möglicher synergistischer Effekt durch wiederholte Inokulationen und Mahd eine Kontrolle der Distel verbessern könnte. Insekten können eine Infektion mit *P. punctiformis* zusätzlich fördern, obwohl mutualistische oder antagonistische Effekte der Rostinfektion auf die Herbivoren von der jeweiligen Art abhängen. Weitere Untersuchungen sind nötig, um *P. punctiformis* und *P. destructiva* als Mykoherbizide zu etablieren und ihren Einfluß auf die Ackerkratzdistel im Ackerbau zu prüfen.

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