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Influence of rust epidemics on interspecific plant competition

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Summary

Epidemiology and the effect of epidemics of the rust fungus *Puccinia lagenophorae* on interspecific competition between the host *Senecio vulgaris* and the non-host *Capsella bursa-pastoris* were studied. The weed-pathogen system *S. vulgaris* - *P. lagenophorae* is a model system used to develop the system management approach of biological weed control. The effects of temperature and plant susceptibility towards the rust fungus were studied in 2 laboratory studies. The effects on life-history characteristics were studied and the results were used to calculate the effects on exponential rate of increase and velocity of focus expansion. The spatial and temporal development of a rust epidemic was determined in a field experiment. In this field experiment the effect of a rust epidemic on competitive balance between *S. vulgaris* and *C. bursa-pastoris* was also determined. The effect of the time a host plant was infected by the rust on the competitive balance was determined in a final field experiment.

The effect of temperature on latent period and aeciospore production of *P. lagenophorae* on *S. vulgaris* was studied in small-scale experiments under controlled conditions (Chapter 2). A clear effect of temperature on latent period was demonstrated. The latent period *p* decreased exponentially with increasing temperature. Both total aeciospore production *I(t)*, and net reproductive number R_0, increased linearly with increasing temperature in a range from 10 to 22°C. Aeciospore production was described by a logarithmic transformation of the gamma density. The mean time to produce an aeciospore, μ, and its standard deviation ν were calculated using the parameters describing the gamma function. The parameters *I(t)*, R_0, *p*, μ and ν were incorporated in models to determine the effect of temperature on epidemic development. The outcome of the models suggested an increase in the exponential growth rate, *r*, and the velocity of focus expansion *c,* with temperature. This increase in epidemic development was mainly caused by the effect of temperature on latent period and on net reproductive number. The effect of the temperature on the sporulation curve seemed to be less important.

The resistance of *S. vulgaris* to *P. lagenophorae* is quantitative, race non-specific and ontogenetic. This type of resistance is often expressed in length of the latent period, which was demonstrated to be an important parameter determining the velocity of epidemic spread. The effect of this resistance on latent period and aeciospore production of *P. lagenophorae* on *S. vulgaris* was therefore studied in small-scale experiments under controlled conditions (Chapter 3). Susceptibility of six plant lines of *S. vulgaris* towards the rust fungus *P. lagenophorae* was determined first. The most susceptible and the most resistant plant lines were pNLd and pUK, collected in the Netherlands and the UK, respectively. Latent period *p*, and aeciospore production *I(t)* were determined for two plant stages of each of these two plant lines grown under two day-night temperature regimes (22-8°C and 22-22°C). Aeciospore production was again well described by a logarithmic transformation of the gamma density. The mean time to produce an aeciospore, μ, and its standard deviation ν were calculated using the parameters describing the gamma function. Velocity of focus expansion *c*, was calculated using estimates of the parameters *p*, μ and ν. Plant line and temperature affected μ and ν, while *p* was affected differently by plant line for each temperature. Calculated velocity of focus expansion was significantly affected by plant line only and was highest on the most susceptible plant line pNLd. The results suggested a of 10 m^2 in focus size between the most susceptible and most resistant plant line after 40 days of expansion of *P. lagenophorae* from one inoculum source.

The models used to determine the temperature and plant line effects on epidemic development assumed focal expansion like a travelling wave. This assumption made it possible to estimate a constant velocity of epidemic spread. The validity of this assumption was determined in the first field experiment in 1998 (Chapter 4). Therefore the spatial and temporal dynamics of an induced rust epidemic on a population of its host *S. vulgaris* grown

in an 8:1 mixture with *C. bursa-pastoris* were quantified in a plot near the University of Fribourg, Switzerland. Both plant species are weeds in agriculture and occur in the same (semi-) natural habitats. The effect of the induced epidemic on the competitive balance between the species was quantified at the end of the experiment. A host plant with sporulating aecia was planted in the centre of the experimental plot. Open aecia on *S. vulgaris* were counted to quantify epidemic spread at regular intervals. A non-random aggregated spatial pattern of open aecia was observed during the whole experiment and a clear focus was formed in the first generation. The spore dispersal gradient (or infection progress curve) was described well by the power law equation at 14 and 28 days after introduction of the inoculum source (a.i.), but not at 32 days a.i. The second generation of the rust epidemic started between 28 and 32 days a.i., and flattened the curve. The infection progress curve described by the power law equation that flattened in time suggested that the epidemic expanded like a dispersive wave with an increasing velocity of spread. Spread of later generations of this type of epidemics is more difficult to predict, than early generations that do not yet differ much from epidemics that spread like a travelling wave. Competitiveness of *C. bursa-pastoris* towards *S. vulgaris* was quantified as total pod production. Competitive balance between the two weeds was not changed in favour of *C. bursa-pastoris* by rust infection. The infection level obtained appeared to be too low to reduce competitive ability of the host at a detectable level. Both time of infection appeared to be too late and the obtained infection level too low, to reduce competitive ability of the host plant towards the non-host. Additionally the initial competitive disadvantage of *C. bursa-pastoris* towards *S. vulgaris* might have been too high to be overcome by a rust epidemic.

Therefore a second field experiment was carried out in 1999 to determine the effect of the time the weed *S. vulgaris* was infected with the rust fungus *P. lagenophorae* on its growth and competitive ability towards *C. bursa-pastoris* (Chapter 5). Four or eight plants of *S. vulgaris* were grown under fertilised and non-fertilised conditions, around one plant of *C. bursa-pastoris*. The first plant species was inoculated once with *P. lagenophorae* 3, 9 or 16 days after transplant or was not inoculated (control). Time of infection with *P. lagenophorae* had a clear effect on growth and development of *S. vulgaris*. This was translated into a small shift in competitive balance for early infections only. A smaller reduction in pod production was observed when *C. bursa-pastoris* was grown between early-inoculated *S. vulgaris* as compared to control plots. Neither fertilisation nor *S. vulgaris* density influenced this effect. This field study showed that the effect of the rust on the performance of its host plant depended on time of infection, fertilisation and plant density. The competitive balance of the host plant towards neighbouring *C. bursa-pastoris* was equally changed by the earliest two infections only and thus depended on time of infection.

The implications for the system management approach of biological weed control of the results presented in this thesis were discussed (Chapter 6). Although the model proposed in the laboratory studies underestimated epidemic development in the field, it can be well used to predict the effects of biotic and abiotic factors on developments of epidemics. Rust epidemics will be induced when inoculum sources are introduced in newly emerging weed populations when temperatures are rising in spring. The advantage of these artificial epidemics is that they develop one or more generations earlier than natural epidemics due to lack of natural inoculum in spring. When also rust strains are introduced that are more aggressive than naturally occurring rust strains towards the local *S. vulgaris* populations these early epidemics are able to reduce development of *S. vulgaris* populations and shift the competitive balance towards the crop.

Zusammenfassung

Die Epidemiologie und der Einfluss von Epidemien des Rostpilzes *Puccinia lagenophorae* auf die interspezifische Konkurrenz zwischen dem Wirt *Senecio vulgaris* und dem nicht-Wirt *Capsella bursa-pastoris* wurden untersucht. Das Unkraut-Pathogen System *S. vulgaris – P. lagenophorae* ist ein Model System welches gebraucht wird um den 'System Management Approach' der biologischen Unkrautbekämpfung zu entwickeln. Die Auswirkungen von Temperatur und Anfälligkeit von Pflanzen auf den Rostpilz wurden in zwei Laborstudien untersucht. Die Einflüsse lebensgeschichtlicher Merkmale wurden erfasst und die Ergebnisse dazu verwendet die Effekte einer exponentiellen Wachstumsrate auf die Zunahme und Geschwindigkeit der fokalen Ausbreitung zu berechnen. Die räumliche und zeitliche Entwicklung einer Rostepidemie wurde in einem Feldexperiment bestimmt. In dieser Feldstudie wurde auch der Einfluß einer Rostepidemie auf die Konkurrenz Bilanz ('competitive balance") zwischen *S. vulgaris* und *C. bursa-pastoris* erfaßt. Die Auswirkung des Zeitpunktes einer Rostinfektion auf die Konkurrenz Bilanz wurde in einer letzten Felduntersuchung ermittelt.

Der Einfluß von Temperatur auf die Latenzzeit und Produktion von Aeciosporen von *P. lagenophorae* auf *S. vulgaris* wurde in klein-schaligen Experimenten unter kontrollierten Bedingungen untersucht (Kapitel 2). Ein deutlicher Effekt von Temperatur auf die Latenzzeit wurde festgestellt. Die Latenzzeit *p* nahm mit zunehmender Temperatur exponentiell ab. Beide, Produktion von Aeciosporen *I (t)* und die netto Reproduktionsrate R_0 nahmen mit zunehmender Temperatur, in einem Bereich von 10 bis 22 °C, linear zu. Die Produktion von Aeciosporen wurde durch eine logarithmische Transformation der Gamma Dichte bestimmt. Die durchschnittliche Zeit μ um eine Aeciospore zu formen, und ihre Standardabweichung ν wurden aus Parametern, welche die Gamma Funktion beschreiben, berechnet. Die Parameter *I(t)*, R_0, μ und ν wurden in die Modelle aufgenommen um den Einfluss von Temperatur auf die Entwicklung der Epidemie zu bestimmen. Das Ergebnis der Modelle weist auf eine Zunahme der exponentiellen Wachstumsrate *r* und der Geschwindigkeit der fokalen Ausbreitung c mit zunehmender Temperatur hin. Die Ausbreitung der Epidemie Entwicklung wurde hauptsächlich durch den Einfluß von Temperatur auf Latenzzeit und netto Reproduktionsrate bewirkt. Der Einfluss von Temperatur auf die Sporulationskurve scheint weniger wichtig zu sein.

Die Resistenz von *S. vulgaris* gegen *P. lagenophorae* ist quantitativ, nicht rassenspezifisch und ontogenetisch. Diese Art von Resistenz kommt oft in der Länge der Latenzzeit zum Ausdruck, welche ein wichtiger Parameter zur Bestimmung der Epidemie Ausbreitungsgeschwindigeit darstellte. Die Auswirkung von Resistenz auf die Latenzzeit und Produktion von Aecidiosporen von *P. lagenophorae* auf *S. vulgaris* wurde daher in klein-schaligen Experimenten unter kontrollierten Bedingungen untersucht (Kapitel 3). Zuerst wurde die Anfälligkeit von sechs *S. vulgaris* Linien auf Befall mit *P. lagenophorae* bestimmt. Die anfälligste und die meist resistente Pflanzenlinie waren jeweils pNLd und pUK, gesammelt in den Niederlanden und England. Die Latenzzeit *p* und die Produktion von Aeciosporen *I(t)* wurden für zwei Pflanzenstadien der beiden Pflanzenlinien erfaßt, welche unter zwei Tag-Nacht Temperatur Systemen (22-8°C und 22-22°C) herangezogen wurden. Die Produktion von Aecidosporen wurde wiederum durch eine logarithmische Transformation der Gamma Dichte bestimmt. Die durchschnittliche Zeit μ um eine Aeciospore zu formen, und ihre Standardabweichung ν wurden aus Parametern, welche die Gamma Funktion beschreiben, berechnet. Die Geschwindigkeit der fokalen Ausbreitung *c* wurde mit Hilfe der Parameter *p*, μ und ν berechnet. Pflanzenlinie und Temperatur hatten einen Einfluss auf μ und ν, während *p* je nach Pflanzenlinie für jede Temperatur unterschiedlich beeinflusst wurde. Die berechnete Geschwindigkeit der fokalen Ausbreitung wurde nur durch die Pflanzenlinie signifikant bestimmt und war in der anfälligsten Pflanzenlinie pNLd am höchsten. Die Ergebnisse weisen auf eine Differenz von $10m^2$ in der Größe des Fokus

zwischen der meist anfälligen und der meist resistenten Pflanzenlinie, 40 Tage nach Ausbreitung von *P. lagenophorae* von der Inokulationsquelle, hin.

Die Modelle, die benutzt wurden um den Einfluss von Temperatur und Pflanzenlinie auf die Entwicklung der Epidemie zu erfassen, gingen von eine fokalen Ausbreitung in Form einer laufenden Welle ('travelling wave') aus. Diese Annahme machte es möglich die konstante Geschwindigkeit der Ausbreitung der Epidemie zu bestimmen. Die Gültigkeit dieser Annahme wurde in einem ersten Feldversuch in 1999 untersucht (Kapitel 4). Hierfür wurden die räumliche und zeitliche Dynamik einer induzierten Rostepidemie in einer Population der Wirtspflanze *S. vulgaris*, welche in einer Mischung von 8:1 mit *C. bursa-pastoris* in einer Versuchsfläche nahe der Universität Fribourg, Schweiz, herangezogen wurde, quantifiziert. Beide Pflanzenarten sind als landwirtschaftliche Unkräuter bekannt und kommen in den gleichen (semi-) natürlichen Lebensräumen vor. Der Einfluss der induzierten Epidemie auf die Konkurrenz Bilanz zwischen den Arten wurden am Ende des Experiments bestimmt. Eine Wirtspflanze mit sporulierenden Aecia wurde in der Mitte der Versuchsfläche angepflanzt. Die Anzahl offener Aecia auf *S. vulgaris* wurde erfaßt um die Ausbreitung der Epidemie quantitativ in regelmäßigen Abständen zu bestimmen. Ein nicht zufälliges aggregiertes räumliches Verbreitungsmuster der offenen Aecia wurde während des gesamten Experiments festgestellt. Ein deutliche Fokus wurde in der ersten Generation gebildet. Der Sporenverbreitungsgradient (oder die Infektionsfortgangskurve) 14 und 28 Tagen, aber nicht 32 Tage nach einbringen der Inokulumsquelle wurde durch eine Potenzgleichung bestimmt. Die zweite Generation der Rostepidemie wurde zwischen 28 und 32 Tagen nach einbringen der Inokulumsquelle gebildet und flachte die Infektionsfortgangskurve ab. Die abflachende Infektionsfortgangskurve, bestimmt durch eine Potenzgleichung, weist auf daraufhin, daß die Epidemie sich wie eine zerstreuende Welle ('dispersive wave') mit zunehmender Ausbreitungsgeschwindigkeit, verbreitet. Die Ausbreitung späterer Generationen ist in dieser Art von Epidemien schwieriger vorher zu sagen als die von als früheren Generationen, welche sich noch nicht zu sehr von Epidemien unterscheiden die sich als laufende Welle verbreiten. Die Konkurrenz von *C. bursa-pastoris* mit *S. vulgaris* wurde als die Gesamtproduktion von Schoten bestimmt. Die Konkurrenz Bilanz zwischen den zwei Unkräutern wurde durch die Rostinfektion nicht zu Gunsten von *C. bursa-pastoris* verändert. Das erzielte Infektionsniveau scheint zu gering zu sein um die Konkurrenzfähigkeit des Wirts auf sichtbare Weise zu reduzieren. Der Zeitpunkt der Infektion ist zu spät und das Infektionsniveau zu niedrig um die Konkurrenzfähigkeit des Wirtes gegenüber dem nicht-Wirt zu vermindern. Zusätzlich kann der anfängliche Konkurrenz Nachteil von *C. bursa-pastoris* gegenüber *S. vulgaris* zu groß gewesen sein um durch eine Rostepidemie überwunden zu werden.

Daher wurde ein zweiter Feldversuch in 1999 durchgeführt um den Einfluß des Infektionszeitpunktes mit *P. lagenophorae* in *S. vulgaris* auf das Wachstum und die Konkurrenzfähigkeit von *S. vulgaris* gegenüber *C. bursa-pastoris* zu untersuchen (Kapitel 5). Vier oder acht Pflanzen von *S. vulgaris* wurden unter gedüngten oder nicht-gedüngten Bedingungen um eine *C. bursa-pastoris* Pflanze herum angeordnet, herangezogen. Nach 3, 9 oder 16 Tagen nach dem Einbringen der Pflanzen wurde *S. vulgaris* mit *P. lagenophorae* inokuliert. Die Kontrollpflanzen wurden nicht inokuliert. Der Infektionszeitpunkt mit *P. lagenophorae* hatte einen deutlichen Einfluß auf das Wachstum und die Entwicklung von *S. vulgaris*. Dieses äußerte sich in einer kleinen Verschiebung der Konkurrenz Bilanz zum frühen Infektionszeitpunkt. Eine geringere Reduktion in der Gesamtproduktion von Schoten wurde festgestellt, wenn *C. bursa-pastoris* zwischen früh infizierten *S. vulgaris*, im Vergleich zur Kontrolle, herangezogen wurde. Weder Düngung noch die Dichte von *S. vulgaris* beeinflußte diesen Effekt. Diese Feldstudie hat gezeigt, daß der Einfluß vom Rost auf die Leistung seiner Wirtspflanze vom Infektionszeitpunkt, der Düngung und der Pflanzendichte abhängt. Die Konkurrenz Bilanz der Wirtspflanze gegenüber der angrenzenden *C. bursa-pastoris* Pflanze war bei den zwei frühen Infektionen in gleicher Weise verschoben und war daher vom Infektionszeitpunkt abhängig.

Die Auswirkungen der erzielten Ergebnisse dieser Doktorarbeit auf den 'System Management Approach' der biologischen Unkrautbekämpfung werden in Kapitel 6 diskutiert. Obwohl das Model, welches in den Laboruntersuchungen erstellt wurde, die Entwicklung der

Epidemie im Feld unterschätzt, kann es dazu eingesetzt werden um den Einfluß von biotischen und abiotischen Faktoren auf die Entwicklung von Epidemien zu bestimmen. Rostepidemien werden induziert wenn Inokulumquellen in neu aufkommenden Unkrautpopulationen im Frühjahr, mit seinen steigenden Temperaturen, eingebracht werden. Der Vorteil dieser künstlichen Epidemien besteht darin, daß sie sich eine oder mehrere Generation früher entwickeln als natürliche Epidemien da natürliche Inokulationsquellen im Frühjahr nicht vorhanden sind. Wenn dabei Roststämme eingebracht werden, welche auf den örtlich vorkommenden *S. vulgaris* Populationen aggressiver sind als die natürlich vorkommenden Roststämme, können diese frühen Epidemien die Entwicklung von *S. vulgaris* Populationen hemmen und die Konkurrenz Bilanz zu Gunsten der Kulturpflanze verschieben.

Samenvatting

De epidemiologie en het effect van epidemieën van de roestschimmel *Puccinia lagenophorae* op interspecifieke competitie tussen de waardplant *Senecio vulgaris* (Klein Kruiskruid) en de niet-waard *Capsella bursa-pastoris* (Herderstasje) zijn bestudeerd. Het onkruid-pathogeen systeem *S. vulgaris - P. lagenophorae* is een model systeem dat wordt gebruikt om de 'system management approach' van biologische onkruidbeheersing te ontwikkelen. De effecten van temperatuur en gevoeligheid van de plant voor de roestschimmel werden bestudeerd in 2 laboratorium studies. De effecten op 'life-history' eigenschappen werden bepaald en de resultaten werden gebruikt om de effecten op exponentiële groeisnelheid (exponential growth rate) en snelheid van focus expansie (velocity of focus expansion) te bepalen. De ontwikkeling in ruimte en tijd van een roestepidemie werden bepaald in een veldexperiment. In dit veld experiment werd het effect van een roestepidemie op de concurrentiebalans (competitive balance) tussen *S. vulgaris* en *C. bursa-pastoris* ook bepaald. Het effect van het moment van infectie door de roest op de concurrentiebalans werd bepaald in een laatste veld experiment.

Het effect van temperatuur op latente periode en aeciospore productie van *P. lagenophorae* op *S. vulgaris* werd bestudeerd in experimenten op kleine schaal onder gecontroleerde omstandigheden (Hoofdstuk 2). Een duidelijk effect van temperatuur op latente periode werd aangetoond. De latente periode *p* nam exponentieel af bij een stijgende temperatuur. Zowel totale sporenproductie *I(t)* als netto reproductie snelheid (net reproduction number) R_0 namen lineair toe met een temperatuurstijging van 10 tot 22°C. Productie van aeciosporen werd beschreven door een logaritmische transformatie van de gamma verdeling. De gemiddelde tijd tot productie van een aeciospore μ en de standaard deviatie ν werden berekend aan de hand van de parameters die de gamma verdeling beschreven. De parameters *I(t)*, R_0, *p*, μ en ν werden in de gebruikte modellen ingevoerd om het effect van temperatuur op epidemische ontwikkeling te bepalen. De uitkomst van de modellen suggereerden een toename in exponentiële groeisnelheid *r* en snelheid van focus expansie *c* met toename in temperatuur. De toename in epidemische ontwikkeling werd voornamelijk veroorzaakt door het effect van temperatuur op latente periode en netto reproductie snelheid. Het effect van temperatuur op de sporulatiecurve leek van minder belang.

De resistentie van *S. vulgaris* tegen *P. lagenophorae* is kwantitatief, niet-rasspecifiek en ontogenetisch. Dit type resistentie komt vaak tot uitdrukking in lengte van de latente periode, waarvan was aangetoond dat het een belangrijke parameter was ter bepaling van de snelheid van focus expansie. Het effect van dit type resistentie op de latente periode en de aeciospore productie van *P. lagenophorae* op *S. vulgaris* werd daarom bestudeerd in experimenten op kleine schaal onder gecontroleerde omstandigheden (Hoofdstuk 3). De gevoeligheid van 6 plantlijnen van *S. vulgaris* tegen de roestschimmel *P. lagenophorae* werd eerst bepaald. De meest gevoelige en meest resistente plantlijnen waren, respectievelijk, pNLd en pUK, verzameld in Nederland en Groot-Brittannië. De latente periode *p* en aeciospore productie *I(t)* werden bepaald voor twee plantstadia van elk van deze twee plantlijnen gekweekt onder twee dag-nacht temperatuur regimes (22-8°C en 22-22°C). Aeciospore productie werd goed beschreven door een logaritmische transformatie van de gamma verdeling. De gemiddelde tijd tot productie van een aeciospore μ en de standaard deviatie ν werden berekend aan de hand van de parameters die de gamma verdeling beschreven. De snelheid van focus expansie *c* werd berekend aan de hand van de geschatte waarden van de parameters *p*, μ en ν. Plantlijn en temperatuur beïnvloedden μ en ν, terwijl *p* voor elke temperatuur verschillend werd beïnvloed door plantlijn. De berekende snelheid van focus expansie werd significant beïnvloed door alleen plantlijn en was het hoogste op de meest gevoelige plantlijn, pNLd. De resultaten suggereerden een verschil van

10 m^2 in focusgrootte tussen de meest gevoelige en meest resistente plantlijn na een 40 dagen durende expansie van *P. lagenophorae* vanuit 1 inoculumbron.

De modellen die werden gebruikt om de effecten van temperatuur en plantlijn op epidemische ontwikkeling te bepalen zijn gebaseerd op focale expansie als een lopende golf (travelling wave). Deze aanname maakt het mogelijk om een constante snelheid van epidemische verspreiding te schatten. De betrouwbaarheid van deze aanname werd bepaald in het eerste veldexperiment in 1998 (Hoofdstuk 4). Daarvoor werden de dynamiek in ruimte en tijd van een geïnduceerde roestepidemie op een populatie van de waard *S. vulgaris*, geplant in een 8:1 mengsel met *C. bursa-pastoris* gekwantificeerd in een proefveld op het terrein van de Universiteit van Fribourg, Zwitserland. Beide plantensoorten zijn onkruiden in landbouw en komen in dezelfde (semi-)natuurlijke habitats voor. Het effect van de geïnduceerde epidemie op de concurrentiebalans tussen de soorten werd gekwantificeerd aan het eind van het experiment. Een waardplant met sporulerende aecia werd in het midden van het proefveld geplant. Open aecia op *S. vulgaris* werden in reguliere intervallen geteld om epidemische verspreiding te kwantificeren. En niet-willekeurig opgehoopt ruimtelijk patroon van open aecia werd waargenomen gedurende het hele experiment en een duidelijke focus werd gevormd in de eerste generatie. De spore verspreidingsgradiënt (of infectie progressie curve) werd goed beschreven door de machtswetverdeling (power law distribution) op 14 en 28 dagen na introductie van de inoculumbron (a.i.), maar niet op 32 dagen a.i. De tweede generatie van de roestepidemie begon tussen de 28 en 32 dagen a.i. en vlakte de curve af. De infectie progressiecurve beschreven door een in de tijd afvlakkende machtswetverdeling suggereerde dat de epidemie zich verspreidde als een uiteendrijvende golf (dispersive wave) met een toenemende verspreidingsnelheid. Verspreiding van latere generaties van dit type epidemieën is moeilijker te voorspellen, dan eerdere generaties die nog niet veel verschillen van epidemieën die volgens een uiteendrijvende golf verspreiden. Concurrentie van *C. bursa-pastoris* tegenover *S. vulgaris* werd gekwantificeerd als totale peulenproductie. De concurrentiebalans tussen de twee onkruiden werd niet veranderd in het voordeel van *C. bursa-pastoris* door de roestinfectie. Het verkregen infectieniveau leek te laag om de concurrentiekracht van waard waarneembaar te reduceren. Zowel moment van infectie leek te laat en het verkregen infectieniveau te laag om concurrentiekracht van de waard tegenover de niet-waard te verlagen. Bovendien kan het initiële concurrentienadeel van *C. bursa-pastoris* tegenover *S. vulgaris* te groot zijn geweest om door de roestepidemie te worden overwonnen.

Daarom werd in 1999 een tweede veldexperiment uitgevoerd om het effect te bepalen van het moment dat het onkruid *S. vulgaris* werd geïnfecteerd door de roestschimmel *P. lagenophorae* op haar groei en concurrentiekracht tegenover *C. bursa-pastoris* (Hoofdstuk 5). Vier of acht planten van *S. vulgaris* werden om een plant van *C. bursa-pastoris* geplant in een bemest of niet-bemest veldje. De eerste soort werd eenmalig geïnoculeerd met *P. lagenophorae* 3, 9 of 16 dagen na het planten of werd niet geïnoculeerd (controle). Het moment van infectie met *P. lagenophorae* had een duidelijk effect op de groei en ontwikkeling van *S. vulgaris*. Dit werd vertaald in een kleine verschuiving in de concurrentiebalans voor enkel de vroege infecties. Een kleinere reductie in peulproductie werd waargenomen wanneer *C. bursa-pastoris* tussen vroeg geïnoculeerde *S. vulgaris* werd geteeld, vergeleken met controleveldjes. Bemesting noch *S. vulgaris*-dichtheid beïnvloedde dit effect. De veldstudie liet zien dat het effect van de roest op de prestatie van haar waardplant afhing van moment van infectie, bemesting en dichtheid. De concurrentiebalans tussen de waardplant en de aangrenzende *C. bursa-pastoris* werd evenveel veranderd door alleen de eerste twee infecties en hing dus af van moment van infectie.

De gevolgtrekkingen voor de 'system management approach' van biologische onkruidbeheersing van de in dit proefschrift gepresenteerde resultaten werden bediscussieerd in hoofdstuk 6. Hoewel het voorgestelde model in laboratoriumstudies de epidemische ontwikkeling in het veld onderschatte, kan het model wel gebruikt worden om de effecten van biotische en abiotische factoren op ontwikkelingen van epidemieën te voorspellen. Roestepidemieën worden geïnduceerd door inoculumbronnen te introduceren in zich nieuw ontwikkelende onkruidpopulaties als de temperatuur in de lente begint te stijgen. Het voordeel van deze kunstmatige epidemieën is dat ze één of meer generaties voorsprong

hebben op natuurlijke epidemieën door gebrek aan natuurlijk inoculum in de lente. Als ook roestlijnen worden geïntroduceerd die agressiever zijn tegen locale *S. vulgaris*-populaties dan de natuurlijke roestlijnen, dan kunnen deze vroege epidemieën de ontwikkeling van *S. vulgaris*-populaties remmen en de concurrentiebalans verschuiven in de richting van het gewas.

Chapter 1

General introduction

WEED CONTROL

Weeds are defined as plants out of place or plants growing where they are not wanted. They can cause crop losses due to competition for resources and can be carriers of pests and diseases. Weed control has been based on the principle of control at any cost since the early days of agriculture (Hurle, 1997). The aim of early weed control was to maintain the level of weed infestation as low as possible in order to prevent yield reduction and a further increase in weed infestation. Due to lack of truly effective weed control methods, a large proportion of farming activity was spent on weeding.

Chemical control

Introduction of chemical herbicides in the early 20th century enabled weeds to be controlled effectively and at a reasonable price. The success of chemical herbicides has further stimulated the idea of crop production in a weed-free environment and up until recently, the clean-crop option has been the ultimate aim in weed control (Müller-Schärer & Frantzen, 1996). The more recent introduction of economic thresholds in weed control optimised the economical use of herbicides. The weed density at which the cost of control equalled the expense of leaving the weeds in the field could now be determined. The farmer did not need to be afraid of being unable to manage an increased infestation in the coming year because he had not controlled his weeds in the previous season (Hurle, 1997; but see: Wallinga & Van Oijen, 1997). Disadvantages as environmental contamination caused by herbicides, difficulties in controlling specific weed species due to herbicide resistance and increasing consumer pressure against all pesticide use have contributed to a re-examination of weed control strategies (Müller-Schärer & Frantzen, 1996).

Biological control

An alternative to chemical weed control is biological control, which is the use of living organisms to control or reduce the population of a weed species (Watson, 1991). Three approaches of biological weed control are considered.

The classical or inoculative approach involves the importation and release of one or more natural enemies into areas where the weed is introduced and is troublesome. These natural enemies attack the target weed in its native range, and are absent in the areas where the weed is troublesome. Its objective is generally not eradication of the weed species, but the self-perpetuating regulation of the weed population at acceptable low levels. This method was for example used to control *Opuntia* spp. by introduction of the moth *Cactoblastis cactorum* in Australia and to control *Alternanthera philoxeroides* (alligatorweed) by introduction of a chrymsomelid beetle in the USA (Hurle, 1997).

The classical approach is less useful for intensively managed agro-ecosystems because reduction of the weed population can take several seasons. Here weeds should be controlled before causing yield loss. More recently, bioherbicides, which are applied as chemical herbicides, have been developed to satisfy the demands for rapid and complete weed control. This inundative or bioherbicide approach relies on the mass production and application of pathogens (Hasan & Ayres, 1990). The plant pathogens used live in association with each plant species but, because of natural constraints, are unable to build up large populations to have a destructive effect. The inundative method of biological control

satisfies the demands for rapid and complete weed control. As for chemical herbicides, there is a need for regular applications using conventional application methods. The inundative approach is used in intensively managed agro-ecosystems. The introduction of weed pathogenic microorganisms should fit in the current agricultural practises. Formulated products of these microorganisms are therefore introduced. Registration of these products is time consuming and costly. Markets are mostly small, and only few well performing microorganisms made it to commercial products (Hurle, 1997).

A third strategy, augmentation, requires periodic re-establishment of a classical biocontrol agent, but to a lesser extent than that required for mycoherbicides (Hasan & Ayres, 1990). Hurle (1997) introduced the term ecological thresholds that take into account not only the costs, but also the benefits of weeds. Increased species diversity is shown to be of ecological importance, for example protection of the soil from erosion and solarization, or of companion plants interfering with pests of the crop (Theunissen, 1994). Biological control agents should therefore not only be seen as weed killers. Non-crop plants will only need to be controlled down to the level where they are no longer the cause of an economically defined negative impact. This may be achieved by infection with a pathogen causing a sub-lethal effect on the weed and by exploiting subsequent reduction of its competitiveness.

Müller-Schärer and Frantzen (1996) redefined the various approaches and proposed a ‘system management’ approach of biological weed control in crops (SMA). This approach is based on the management of a weed pathosystem in order to maximise the natural spread and disease severity of a native or naturalised pathogen. It excludes disruptive events, such as the introduction of exotic control organisms (classical approach) or the mass release of inoculum (inundative approach). Its aim is not to eradicate plant species but to shift the balance in competitive weed-crop interactions in favour of the crop. In this regard, biological control agents must be seen as stress factors, not as weed killers, and biological weed control as an integral part of a well-designed pest management strategy, not as a cure by itself.

The SMA may be well suited for control of single weed species in a crop, or areas where no immediate and complete control is required. The potential weed control microorganism can not be formulated as mycoherbicide. Production of large amounts of the microbial control agent is limiting, as it is e.g. a biotrophic fungus. The SMA may also be well suited in areas where importation of an exotic agent is not possible, for example in natural reserves. The success of the SMA depends on the fast development of a plant pathogenic epidemic and the effect of this epidemic on the competitive balance between weed and crop.

OBJECTIVES

The weed pathosystem of *Senecio vulgaris* and *Puccinia lagenophorae* is used as the study system to develop the system management approach of biological weed control further. The weed *Capsella bursa-pastoris* has been used as competitive plant species to model the effect of an epidemic on interspecific competition in this thesis. *Senecio vulgaris* and *C. bursa-pastoris* are known competitors and *P. lagenophorae* is known to affect the competitive balance between the two weeds. The objectives of the present thesis were: i) to quantify the effects of abiotic and biotic factors on velocity of expansion of *P. lagenophorae* epidemics and ii) to quantify the effects of induced *P. lagenophorae* epidemics on the competitive balance between its host *S. vulgaris* and the non-host *C. bursa-pastoris*

STUDY ORGANISMS

Senecio vulgaris

Senecio vulgaris (Asteraceae), common groundsel, is a member of the community of waste ground, arable and garden weeds in Central Europe. It is a self-compatible and strongly self-pollinating annual plant species originating from southern Europe (Kadereit, 1984). *S. vulgaris* can produce up to 3 generations per year. Plants may survive winter vegetatively and start seed production in spring. The soil seed bank of *S. vulgaris* is of minor importance due to nearly complete absence of dormancy and short seed survival in soil. Development of a new generation of *S. vulgaris*, therefore, depends strongly on seedling establishment. A new generation will start to build-up only when plant coverage decreases, *e.g*. when senescence of adult plants starts, since germination is strongly dependent on availability of light (Popay & Roberts, 1970; Roberts & Feast, 1972). Plant size and reproductive capacity of *S. vulgaris* are strongly affected by nutrient availability. Populations of *S. vulgaris* in fertilised agricultural sites are therefore generally denser than in ruderal populations (Leiss & Müller-Schärer, 2001a).

In general, *S. vulgaris* is not a major weed as indicated by the result of a European weed survey (Schroeder *et al*., 1993). Plants of this species may however cause problems as yield reduction due to competition with *S. vulgaris* has been demonstrated for lettuce (Paul & Ayres, 1987a) and tomatoes (Qasem & Hill, 1994). Thus, *S. vulgaris* has the potential to become a problem if not controlled, especially in horticulture where frequent cultivations occur (Paul *et al,* 1993). Resistance to herbicides hampers successful control of the weed. Herbicide resistant populations of *S. vulgaris* are now common and widely distributed, especially in Europe and North America (Holt and LeBaron, 1990). Biological control might be an alternative to the chemical strategy due to this presence of herbicide-resistant plants.

Puccinia lagenophorae

Two species of rust fungi were reported by Gäumann (1959) to infect *S. vulgaris*: *Coleosporium senecionis* (Persoon) Fries and *Puccinia senecionis-acutiformis* Hasler, Mayor et Cruchet. Wilson & Henderson (1966) reported *Coleosporium tussilaginis* (Pers.) Lév., which encompasses *C. senecionis* and *Puccinia lagenophorae* Cooke as rust fungi infecting plants of *S. vulgaris.* The autoecious rust fungus *P. lagenophorae* (Uredinales, Basidiomycetes), is the most prevalent pathogen of *S. vulgaris* in Europe nowadays (Frantzen & Hatcher, 1997; Wyss & Müller-Schärer, 1999), capable of rapid disease build-up and high levels of infection. *Puccinia lagenophorae* is understood to be native on *S. vulgaris* in Australia spreading to Europe in the early 1960s (Viennot-Bourgin, 1964). The rust fungus colonises leaves, stems and capitula of *S. vulgaris* by way of aeciospores and reduces development of the plant. Although teliospores are produced, this spore stage does, so far known, not play a role in the infection cycle. Plants seem to be infected only by way of aeciospores in Europe (Wilson *et al*., 1965). Leaf area, vegetative biomass, number of capitula and reproductive biomass of infected *S. vulgaris* are reduced (Leiss & Müller-Schärer, 2001b). Infection by the rust increases the host's vulnerability to environmental stress (Paul & Ayres, 1984; 1986a; 1986b; 1986c; 1987b).

The rust is known to reduce the competitive ability of *S. vulgaris* towards crops and other weeds (Paul & Ayres, 1987a; 1990; Paul, 1989; Frantzen, 2000; Grace & Müller-Schärer, 2003). It has therefore been proposed as a potential biological control agent of the weed (Müller-Schärer & Frantzen, 1996; Frantzen & Hatcher, 1997). Its applicability as biocontrol agent is however limited due to the absence of *P. lagenophorae* epidemics to reduce growth, reproduction and survival of *S. vulgaris* in spring. This absence is mainly due to low winter survival of the pathogen. *Puccinia lagenophorae* overwinters as mycelium in groundsel plants, infected in autumn, but equally causes high winter mortality of especially small host plants. Therefore, surviving plants are nearly free of rust in spring (Paul & Ayres, 1986a; 1986b; Frantzen & Müller-Schärer, 1999). As a result, only few inoculum sources are

available in spring and it takes until summer before natural epidemics develop (Leiss & Müller-Schärer, 2001b). Adverse climatic conditions may hamper rust development in spring further, though very little quantitative information is available. Additionally, younger stages of *S. vulgaris* were found to be less susceptible to *P. lagenophorae* infection, than older stages, which may further reduce the effect of the rust on the population dynamics (Wyss & Müller-Schärer, 1999; Leiss & Müller-Schärer, 2001b).

Although rust incidence levels increase strongly after *S. vulgaris* populations start to increase again, the population dynamics of the plant seems hardly affected by the rust. The high disease pressure appears to be initiated too late (Leiss & Müller-Schärer, 2001b).

Capsella bursa-pastoris

The non-host *Capsella bursa-pastoris* (Brassicaceae) is also a member of the community of waste ground, arable and garden weeds in Central Europe. *Capsella bursa-pastoris*, shepherd's purse, is a self-compatible and strongly self-pollinating annual plant species. The basal leaves form a rosette that can overwinter, depending on plant size and winter weather. The stem elongates at flowering. Seeds are produced in pods (silicula). First ripe seeds can already be produced within a month after seed germination. Seed production can continue for several months. The number of seeds per pod and number of pods per plant depend on genotype and environment (Aksoy *et al*., 1998).

Competition between *Senecio vulgaris* and *Capsella bursa-pastoris*

Competition can be defined as the active demand by two or more organisms for a resource, so that both are inhibited by the demand, e.g. plants competing for light or water. (Lawrence, 1995). Begon *et al*. (1990) defined competition more specific as the interaction between individuals, brought about by shared requirement for a resource in limited supply, and leading to a reduction in the survivorship, growth and/or reproduction of the competing individuals concerned.

Direct competition for space and light between two neighbouring plants does not start before the available surface is covered and plants are large enough to withhold light from their neighbours. Therefore competition for light and space will start earlier in high density populations, with plants growing close to each other, than in low density populations. Early germination, fast growth and development, plasticity to environmental changes and fast recovery can improve a plant's initial advantage over its competitors. Competition can occur between individuals of the same (intraspecific competition) and different species (interspecific competition).

The clean crop option of weed control in agriculture equals a complete exclusion of interspecific competition between crop and weeds by removal of the weed. The system management approach of biological weed control is based on reduction of competitive ability of weeds towards crops. Weeds are allowed in a crop as long as the competitive balance is in favour of the crop (Müller-Schärer & Frantzen, 1996).

The competitive balance between *C. bursa-pastoris* and *S. vulgaris* depends on availability of nutrients. *S. vulgaris* has a competitive advantage over *C. bursa-pastoris* under nutrient-rich, but not under nutrient-poor conditions. Infection of *S. vulgaris* with *P. lagenophorae* eliminates the advantage of the host over *C. bursa-pastoris* (Paul & Ayres, 1990).

Epidemiology of *Puccinia lagenophorae*

Epidemics of *P. lagenophorae* have to develop from a few, small, inoculum sources in spring. An infected plant may act as a primary focus inside a plant population. The disease may subsequently spread rotationally symmetric, as the focus expands by reproduction and dispersal of the pathogen. The disease can spread as a travelling wave with constant velocity, or as a dispersive wave with continuously increasing velocity (Frantzen & Van den Bosch, 2000). The spatial distribution of the released fungal spores determines the wave

type of spread (Kot *et al*., 1996). Offspring settles down at a distant place from the mother. This distance is called the dispersal distance. The probability density function describing the dispersal distance of all offspring is called the contact distribution for pathogens (Van den Bosch *et al*., 1988a). If the curve can be fitted with an power law distribution, the disease will spread with a travelling wave. If an exponential function fits the distribution best, the disease will spread with a dispersive wave.

The constant velocity of increase of the radius of the area covered by a pathogen of a travelling wave can be predicted using an analytical model (Van den Bosch *et al.*, 1988a; 1988b; Frantzen & Van den Bosch, 2000). The intensity and extent of an epidemic depend on the effective multiplication factor of a pathogen, the latent period and the infectious period. The effective multiplication factor, R_0, may be defined as the number of lesions produced per mother lesion per day. The latent period p, may be defined as the period between penetration of the host by a pathogen and the start of production of dispersal units on the host, and determines the delay in time in the spread of a pathogen. The infectious period may be defined as the period of production of dispersal units.

Epidemic development depends on both abiotic (*e.g.* climatic conditions) as biotic (*e.g.* pathogenicity and the host's resistance) factors. Latent period, lesion development and sporulation depend in general on temperature, humidity, leaf wetness duration and solar radiation rates (Zadoks & Schein, 1979). Several authors also mention the effect of temperature before infection on epidemic parameters (Brown & Shipton, 1964; Ramage & Sutherland, 1995; Gijzen *et al.*, 1996). Quantitative resistance has often been revealed in a plant line or cultivar effect on latent period (Parlevliet, 1975; Broers, 1989; Wilson & Shaner, 1989; Lehman & Shaner, 1996; Chongo & Bernier, 1999). Interactions between environment and plant lines with respect to quantitative resistance are likely to occur (Jenns & Leonard, 1985; Shaw, 1990; Figueroa *et al*., 1995; Carisse & Peyrachon, 1999).

The net reproduction number R_0 of *P. lagenophorae* on *S. vulgaris* was estimated at 383 and the contact distribution could be described by a double exponential function with a standard deviation σ of 28 cm (Frantzen & Van den Bosch, 2000). The shortest known latent period p of *P. lagenophorae* on *S. vulgaris* is 10 days under controlled, optimum conditions (Paul & Ayres, 1984). Latent period lasted 14 days in a field experiment in spring (Frantzen & Müller-Schärer, 1998). Resistance of *S. vulgaris* to *P. lagenophorae* is quantitative, race non-specific and ontogenetic (Wyss & Müller-Schärer, 1999). Differences in resistance of *S. vulgaris* lines to the rust may therefore be revealed in latent period and infectious period.

OUTLINE

The effect of the abiotic factor temperature on the velocity of epidemic spread of the rust *P. lagenophorae* on its host *S. vulgaris* was quantified in chapter 2. In chapter 3 the effect of the biotic factor resistance on the velocity of epidemic spread was quantified using one rust strain on two plant lines of *S. vulgaris*. In both chapters the epidemic spread was assumed to be focal in order to be able to estimate the velocity of spread. A field study was carried out to determine the type of epidemic spread of *P. lagenophorae* on a *S. vulgaris* population (chapter 4). The host plant was grown in an 8:1 mixture with *C. bursa-pastoris*. The effect of the induced epidemic on competitiveness of *S. vulgaris* towards its competitor could therefore be quantified in the same field experiment. A final field study was carried out to quantify the effect of time of rust infection, groundsel density and fertilisation on competitive balance between *S. vulgaris* and *C. bursa-pastoris* (chapter 5). The results and their implications for the further development of the system management approach of biological weed control are discussed in chapter 6.

Chapter 2

The effect of temperature on epidemiological parameters of *Puccinia lagenophorae*.

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We determined the effect of temperature on latent period and aeciospore production of *P. lagenophorae* on *S. vulgaris* in small-scale experiments under controlled conditions. A clear effect of temperature on latent period was demonstrated. Latent period decreased exponentially with increasing temperature. Both total aeciospore production and net reproductive number increased linearly with increasing temperature in a range from 10 to 22°C. The three parameters were incorporated in models to determine the effect of temperature on epidemic development. The present study suggests an increase in the exponential growth rate, *r*, and the velocity of focus expansion *c,* with temperature. This increase in epidemic development was mainly caused by the effect of temperature on latent period and on net reproductive number. The effect of the temperature on the sporulation curve seemed to be less important.

INTRODUCTION

Senecio vulgaris (Asteraceae) is a self-compatible and strongly self-pollinating annual plant species originating from southern Europe (Kadereit, 1984). It is a significant weed, especially in horticulture where frequent cultivations occur (Paul *et al,* 1993). The rust fungus *Puccinia lagenophorae* (Basidiomycetes: Uredinales) infects *S. vulgaris* and is known to reduce the competitive ability of the weed towards crops and other weeds (Paul & Ayres, 1987a; Paul & Ayres, 1990). It therefore has been proposed as a potential biological control agent of the weed (Frantzen & Hatcher, 1997; Müller-Schärer & Frantzen, 1996). The rust fungus colonises leaves, stems and capitula of *S. vulgaris*. Although teliospores are produced, this spore stage does not play a role in the infection cycle. Plants seem to be infected only by way of aeciospores in Europe. *P. lagenophorae* probably originates from Australia and was observed throughout the United Kingdom and Europe in the early 1960's (Mayor, 1962; Viennot-Bourgin, 1964; Wilson *et al*., 1965).

In the field, *P. lagenophorae* infections are most severe in late summer and autumn, but sometime, infected seedlings are found in spring (Müller-Schärer & Frantzen, 1996). The absence of sufficient epidemic levels to reduce growth, reproduction and survival of *S. vulgaris* in spring limits its applicability as biocontrol agent. The limited disease levels in spring are due to two factors. First and most important is the low through winter survival of the pathogen. *P. lagenophorae* survives as mycelium within overwintering host plants (Frantzen & Müller-Schärer, 1999). Overwintering of *S. vulgaris* is infrequent however since it is an annual plant. Moreover, survival of *S. vulgaris* plants in winter is greatly reduced by *P. lagenophorae* (Paul & Ayres, 1986a; 1986c; Frantzen & Müller-Schärer, 1999). Therefore, from the epidemic the previous year only a limited amount of sources of inoculum survives to start an epidemic the next spring. The second cause of low epidemic severity in spring is found in adverse climatic condition, though very little quantitative information is available for *P. lagenophorae*. In most fungal plant diseases infection, latent period, lesion development and sporulation depend on temperature, humidity, leaf wetness duration and solar radiation rates (Zadoks & Schein, 1979). Several authors also mention the effect of pre-inoculation temperature on epidemic parameters (Brown & Shipton, 1964; Gijzen *et al.*, 1996; Ramage & Sutherland, 1995). The effect of climatic variables on *P. lagenophorae* and its epidemics is

not well studied. Therefore, we will, in this paper, study the effects of temperature on epidemic parameters of this disease.

The study presented approaches the problem in two steps. First, we will study the effect of temperature on life-history characteristics of the individual infections. Experiments are done to assess the effect of

1. post-infection temperature on the latent period
2. post-infection temperature on total number of spores produced
3. post-infection temperature on the sporulation curve
4. possible interactions of pre- and post-infection temperature on these epidemic parameters.

Secondly, we will study the effect of temperature on epidemic development. An experimental approach to these population level effects of temperature would need large scale, multi-year field experimentation. Such field experiments are presently not available and we take recourse to calculating epidemic development parameters on basis of our measurements at the individual level. We will study the effect of temperature on

1. the exponential rate of increase, *r*, using the Euler equation (Roughgarden, 1979)
2. the velocity of focus expansion *c,* using the model developed by van den Bosch *et al.* (1988a; 1988b).

Both models have gone beyond the theoretical-construct-stage and are intensively used by ecologists and epidemiologists (Buiel *et al*., 1989; Van den Bosch *et al*., 1988c; Van den Bosch *et al*., 1992). Both model types have been extensively verified (Levin, 1989; Minogue & Fry, 1983a; 1983b; Van den Bosch *et al*., 1988c; Van den Bosch *et al*., 1990a; Van den Bosch *et al*., 1990b; Zawolek & Zadoks, 1992; Zadoks & Van den Bosch, 1994).

MATERIAL AND METHODS

Origin of plants and rust

Plants of *S. vulgaris*, line ELS 1 originate from a plant collected in Unterehrendingen (Switzerland) in 1993. Selection and cultivation of *S. vulgaris* lines is described by Wyss (1997). Seeds used in the experiments are the fourth generation of line ELS 1 and were collected between 23-9-1997 and 15-10-1997 from plants grown in a climate chamber at the University of Fribourg. Seeds were stored in paper bags until use.

Aeciospores of *P. lagenophorae*, strain ELS, used in this experiment originate from a single-sorus isolate of *P. lagenophorae*, collected from *S. vulgaris* in an organic seedling cultivation at Unterehrendingen (Switzerland). The isolate was collected in 1993 and cultivated since as described by Wyss (1997).

Plant production: Seeds were germinated in shallow trays filled with nutrient amended peat (Floragard TKS 2), placed in incubators with a day temperature of 10, 16, or 22°C and a night temperature of 8°C, a 16h photoperiod and relative humidity fluctuating between 70 and 80%. Seedlings at the second and third leaf stage were transplanted to 9-cm diameter plant pots (one plant per pot) and returned to the incubators. Plants grown for determination of spore production were directly sown in 9-cm pots, grown under conditions described above and thinned to one plant per pot before inoculation, in a separate experiment. The temperature treatments were randomly allocated over the incubators in each experiment.

Pre-inoculation temperature: To test whether the temperatures at which the plants are grown before inoculation affects epidemiological parameters, three pre-inoculation temperatures were used; 10, 16 and 22°C. Plants grown at these pre-inoculation temperatures will be referred to as group I, group II and group III, respectively. From each of these groups, equal numbers of plants will be used at the various post-inoculation temperatures.

Latent period

Inoculation: Plants of each group were inoculated when 50% of the plants had on average three fully developed leaves (44, 29 and 25 days after sowing, for group I, II and III, respectively). Aeciospores were evenly distributed over *S. vulgaris* plants using a settling tower. The plants were removed from the settling tower and ten plants from each group were grown at post-inoculation temperatures of 10, 13, 16, 19 or 22°C during the day and 8°C at night. Immediately after inoculation plants were covered with plastic for 15 hours to provide the high humidity needed for infection.

Assessment: Plants were examined for presence of open aecia every day. Latent period was determined for each plant as the number of days between inoculation and presence of the first open aecium.

Data analysis: The data on latent period were analysed with pre-inoculation temperature as factor, in which group I, II and III represent a pre-inoculation temperature of 10, 16 and 22°C, respectively. The effect of pre-inoculation temperature on latent period was tested on latent period by means of analysis of variance (ANOVA). An exponential function relating post-inoculation temperature, T in °C, and latent period $p(T)$ in days, was fitted to the data,

$$p(T) = f * e^{-gT} \quad (1)$$

where f and g are shape parameters.

Spore production

Inoculation: Plants from group I, II and III were inoculated 70, 40, and 28 days after sowing, respectively, in a separate experiment as described above. Ten plants from each group were placed at either 10, 16, or 22°C during the day and 8°C at night, after inoculation.

Assessment: Aeciospore production was determined by collecting aeciospores from each individual plant every two days after the latent period. Aluminium foil (9*9 cm) was placed below the first leaf pair when the first symptoms appeared. Flowers were removed from the plants to prevent contamination of collected aeciospores with pappus and seed. Aeciospores were collected by brushing them from the leaves into a snapcap bottle with a paintbrush. Aeciospores on the foil were collected and added to the bottle. The number of aeciospores collected was assessed using a hemacytometer. Five ml demineralised water and two drops of Tween 20 were added to each bottle. The bottles were shaken until all aeciospores were suspended in the solution. An aliquot (0.064 µl) was placed in a hemacytometer and all aeciospores present were counted. Ten aliquots were sampled from each snapcap bottle. Aeciospore collection was stopped when no visible aeciospore production occurred.

Data analysis: For each pre- and post-inoculation temperature combination, a gamma density (Mood *et al.*, 1974) was used to describe aeciospore production $I(t)$,

$$I(t) = I_{tot} \frac{\beta(\beta t)^{\eta-1} e^{(-\beta t)}}{\Gamma(\eta)} \quad (2)$$

where $\Gamma(\eta)$ is the Gamma function, I_{tot} is the total number of aeciospores produced, and η and β are constants of the gamma density with dimension 1 and T^{-1}, respectively. After logarithmic transformation equation (2) becomes:

$$y = \alpha + \delta_1 x_1 + \delta_2 x_2 \quad (3)$$

where $y = \ln(I)$, $x_1 = \ln(t)$, $x_2 = t$, $\alpha = \ln[I_{tot}\beta^{\eta}/\Gamma(\eta)]$, $\delta_1 = \eta - 1$, $\delta_2 = -\beta$. The mean of the gamma density μ can be interpreted as the mean time required to produce a randomly selected spore after the latent period. In terms of the parameters of the gamma density,

$$\mu = \eta / \beta = (\delta_1 + 1) / -\delta_2 \quad (4)$$

The standard deviation of the gamma distribution, ν, is interpreted as the standard deviation of the time required to produce a randomly selected spore. In terms of the parameters of the gamma density,

$$\nu = \sqrt{\eta / \beta^2} = \sqrt{(\delta_1 + 1) / -\delta_2^{\,2}} \quad (5)$$

Parameters α, δ_1 and δ_2 were estimated using linear least-squares regression on the means for each pre- and post-inoculation temperature combination.

The net reproductive number R_0, depends on total spore production per lesion, I_{tot} in equation (2), and infection efficiency of a spore. Assuming that infection efficiency of aeciospores of *P. lagenophorae* under optimal humidity conditions is not affected by temperature, the net reproductive number R_0, and the total aeciospore production per plant, I_{tot}, are linearly related, $R_0 = k * I_{tot}$, where k is a calibration constant. Total number of aeciospores produced was determined for each pre- and post-inoculation temperature combination. A linear relation between total aeciospore production I_{tot}, and temperature was fitted to the data, $I_{tot} = a + bT$, in which T is the average temperature (°C) and a and b are parameters. The total number of new aecia caused by one aecium during the entire sporulation period, the net reproductive number R_0, is then given by $R_0 = k(a + bT)$. To calibrate this relation a net reproductive number of 383 in a field of *S. vulgaris* at an average temperature of 16.33°C (Frantzen & Van den Bosch, 2000) was used. With this we arrive at:

$$R_0 = \frac{383(a + bT)}{(a + 16.33b)} \quad (6)$$

The net reproductive number was calculated for each pre- and post-inoculation temperature combination, using the average of temperature during day and night.

Calculating the exponential growth rate and the velocity of focus expansion

In the initial phase of an epidemic, with few lesions in a virtually uninfected population the number of lesions will increase exponentially in time with a rate known as the exponential growth rate, r. The exponential growth rate in an age-structured population is given by:

$$r \approx \left(\frac{1}{(p + \mu)}\right) * (\ln R_0) * (1 + \kappa * (\ln R_0)) \quad (7)$$

where:

$$\kappa = 0.5 * \left(\frac{\nu}{(p + \mu)}\right)^2 \quad (8)$$

This formula is an extended version of the well known relation $r \approx \ln(R_0)/(p+\mu)$ often used in entomology and first derived from the Lotka-integral-equation for an age-structured population (Roughgarden, 1979) by Keyfitz (1968). The exponential growth rate, r, was calculated for each pre- and post-inoculation temperature combination. The value of p was calculated from equation (1).

The velocity of focus expansion c, expressed in centimetre per day, was calculated for each pre- and post-inoculation temperature combination using equation (A.1) from Van den Bosch *et al*. (1988b):

$$\begin{cases} \ln(R_0) - \ln(1 - \tfrac{1}{2}\lambda^2) - \lambda c^* p^* - \alpha \ln\left(1 + \dfrac{c^* \lambda}{\alpha}\right) = 0 \\ \dfrac{\lambda}{1 - \tfrac{1}{2}\lambda^2} - c^* p^* - \dfrac{c^*}{\dfrac{c^* \lambda}{\alpha} + 1} = 0 \end{cases} \quad (9)$$

where λ is a shape parameter of the wave front, $\alpha = \beta^2$, $c^* = c\eta/\beta\sigma$ and $p^* = p\beta/\eta$. For this calculation the standard deviation of the spatial distribution of daughter lesions around a mother lesion, σ, has to be measured. This parameter was not determined in the present experiment. The velocity of focus expansion is however linearly dependant on the parameter σ (Minogue & Fry, 1983a; Van den Bosch *et al.*, 1988a). Therefore, we will express the velocity as:

$$c' = c/\sigma. \qquad (10)$$

RESULTS

Latent period

The latent period decreased with increasing post-inoculation temperature (Figure 1). No significant (ANOVA, P= 0.68) effect of pre-inoculation temperature on latent period was demonstrated and an exponential curve was fitted through the combined data set. Fitting the equation $p(T) = f * e^{-gT}$ to the data resulted in estimates of f = 40.32 (standard error of 0.70) and g = 0.058 (standard error of 0.001) and the R^2 was 0.95. The expected length of the latent period at a post-inoculation temperature of 10, 16 and 22°C based on the values of f and g, was 22.6, 15.9 and 11.3 days, respectively.

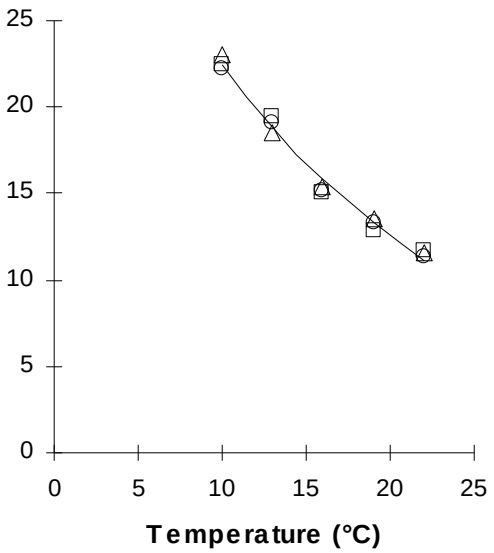

Figure 1. Relation between latent period and post-inoculation temperature. Symbols are measurements from group I, pre-inoculation temperature of 10°C (Δ), means of 6-10 plants, group II, pre-inoculation temperature of 16°C (◊), means of 8-10 plants and group III, pre-inoculation temperature of 22°C (O) means of 9-10 plants. Continuous line is best fitted equation, $p(T) = 40.32 * e^{-0.058T}$.

Spore production

Aeciospore production was well described by the gamma distribution (Figure 2). The curves underestimated the maximum aeciospore production, but standard errors of the estimated parameter values were small and, except for a post-inoculation temperature of 10°C in group II, R^2 was higher than 0.85 (Table 1). Aeciospore production was not affected by pre-inoculation temperature (Figure 2). The mean, μ, and standard deviation, ν, of the time required to produce a randomly selected spore after the latent period decreased with increasing post-inoculation temperature in all groups (Table 1). Total aeciospore production

per plant increased with increasing post-inoculation temperature in all but one group (Table 2). The total aeciospore production at a post-inoculation temperature of 22°C was very low in group I. Regression analyses of the relation between average post-inoculation temperature and total aeciospore production I_{tot}, resulted in estimates of a=0 and b= 1.43*10^6 (standard error of 2.45*10^5) and the R^2 = 0.81, and calibration factor k=1.64*10^{-5}. The net reproductive number increased with increasing post-inoculation temperature in all but one group (Table 2).

Table 1. Estimates and standard error (in parentheses) of parameters of a gamma function[a] fitted to the data of aeciospore production of *P. lagenophorae* on *S. vulgaris* for different post-inoculation temperatures.

Pre-inoculation temperature group	Post-inoculation temperature	δ_1		δ_2		R^2	μ[b]	ν[c]
I (10°C)	10	3.20	(0.25)	-0.14	(0.01)	0.85	30.0	14.6
	16	2.20	(0.20)	-0.15	(0.01)	0.91	21.3	11.9
	22	2.20	(0.26)	-0.18	(0.01)	0.93	17.8	9.9
II (16°C)	10	1.73	(0.37)	-0.09	(0.02)	0.52	30.2	18.3
	16	3.21	(0.16)	-0.24	(0.01)	0.97	17.5	8.5
	22	2.58	(0.22)	-0.26	(0.01)	0.98	13.8	7.3
III (22°C)	10	1.75	(0.28)	-0.13	(0.01)	0.86	21.2	12.8
	16	2.28	(0.25)	-0.16	(0.01)	0.89	20.5	11.3
	22	2.21	(0.35)	-0.22	(0.02)	0.91	14.6	8.1

[a] Gamma function fitted in the form of $y = \alpha + \delta_1 x_1 + \delta_2 x_2$ (See equation 3 in text). [b] Mean time required to produce a randomly selected aeciospore after the latent period has ended, $\mu = \frac{\delta_1 + 1}{-\delta_2}$. [c] Standard deviation of aeciospore production $\nu = \sqrt{\frac{\delta_1 + 1}{(-\delta_2)^2}}$.

Table 2. Estimates and standard error (in parentheses) of total aeciospore production $I_{tot,}$ and calculated net reproductive number R_0, of *P. lagenophorae* on *S. vulgaris* for different post-inoculation temperatures.

Pre-inoculation temperature group	Post-inoculation temperature	Total aeciospore production I_{tot}(*10^6)		Net reproductive number R_0[a]
I (10°C)	10	9.85	(7.0)	161.1
	16	27.14	(8.4)	443.9
	22	9.21	(2.2)	150.6
II (16°C)	10	0.76	(0.4)	12.4
	16	23.91	(11.0)	391.1
	22	33.74	(11.0)	551.9
III (22°C)	10	2.55	(1.3)	41.7
	16	20.19	(10.0)	330.2
	22	35.45	(1.1)	579.8

[a] $R_0 = 1.64 * 10^{-5} * I_{tot}$; see text for explanation.

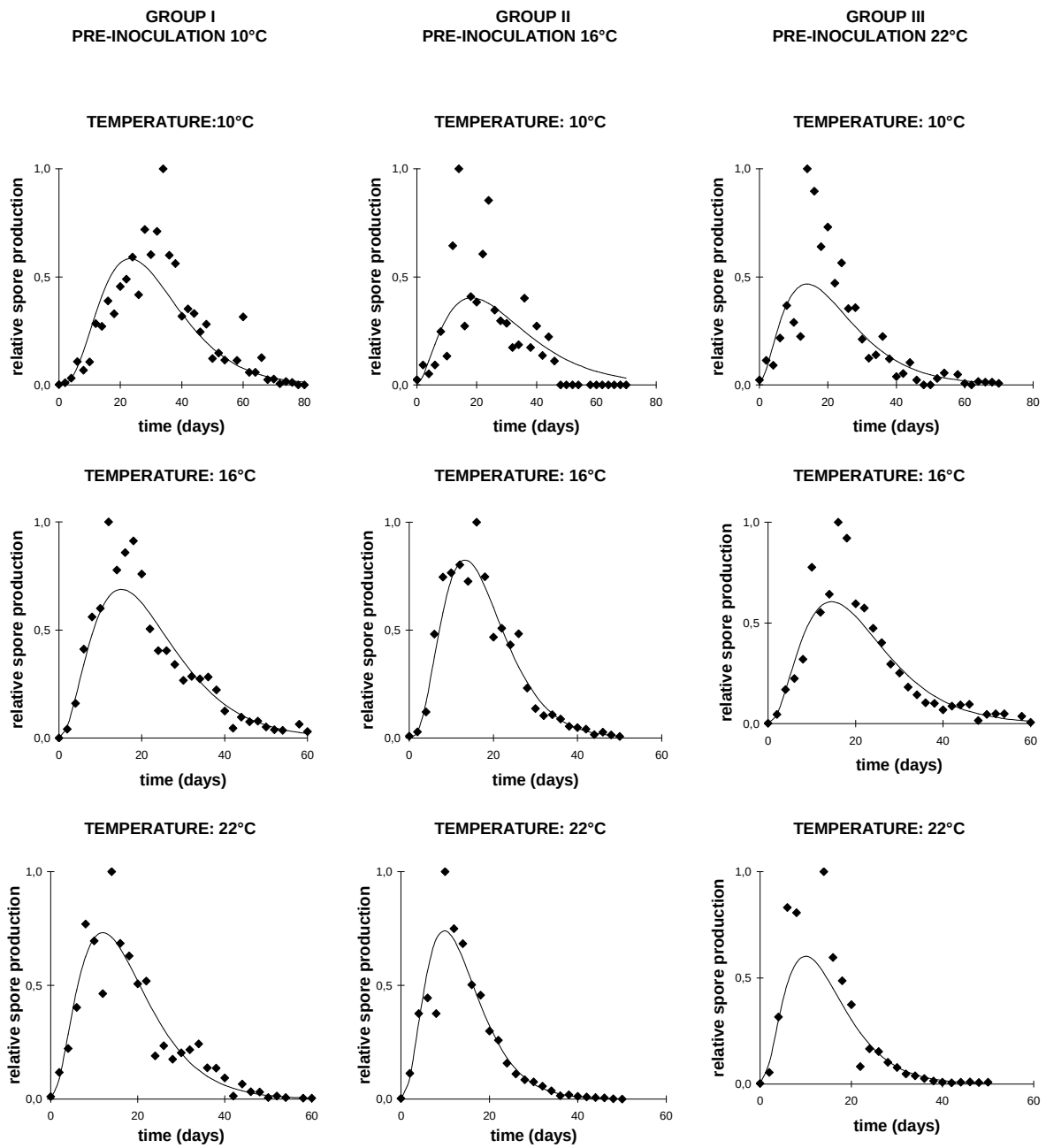

Figure 2. Relative spore production per plant per day of *Puccinia lagenophorae* on *Senecio vulgaris*, determined as fraction of maximum spore production per plant. Dots in group I are means of 10, 8 and 5 plants for 22°C, 16°C and 10°C, respectively. Dots in group II are means of 10, 7 and 6 plants for 22°C, 16°C and 10°C, respectively. Dots in group III are means of 9, 7 and 7 plants for 22°C, 16°C and 10°C, respectively. Continuous curves are best fitted gamma densities. Time 0 indicates the end of the latent period and the start of spore production.

Exponential growth rate and velocity of focus expansion

The exponential growth rate increased by a factor 3 when temperature increased from 10 to 22°C (Table 3). Velocity of focus expansion, expressed as *c'* also increased with increasing post-inoculation temperature (Table 3). At 22°C the velocity is about 3 times as fast as at 10°C. In spite of the low value of R_0 at 22°C in group I, values of both *c'* and *r* also increased with increasing temperature in group I. This increase of both *c'* and *r* was caused by the temperature effect on latent period.

Table 3. Estimates of relative velocity of focus expansion *c',* and exponential growth rate, *r*, of *P. lagenophorae* on *S. vulgaris* for different post-inoculation temperatures.

Pre-inoculation temperature group	Post-inoculation temperature	Relative velocity of focus expansion c' (cm cm^{-1} day^{-1})[a]	exponential growth rate, r (lesion $lesion^{-1}$ day^{-1})
I (10°C)	10	0.12	0.11
	16	0.22	0.22
	22	0.23	0.22
II (16°C)	10	0.07	0.06
	16	0.23	0.22
	22	0.31	0.31
III (22°C)	10	0.11	0.10
	16	0.21	0.21
	22	0.32	0.32

[a] $c' = c/\sigma$ where *c* is the velocity of focus expansion in cm per day and σ is the standard deviation of the spore dispersal distribution.

DISCUSSION

A clear effect of temperature on the latent period of *P. lagenophorae* was demonstrated. The confounding effect of incubators appeared to be negligible because the relationship between temperature and latent period was almost equal on both plants grown for determination of the latent period and for determination of spore production (data not presented). The use of five temperatures to determine the relationship between temperature and latent period enabled a reliable fit. In the temperature range between 10 and 22°C the latent period decreased exponentially with temperature. As discussed by several authors the relationship between latent period and temperature is often asymmetric U-shaped (Shearer & Zadoks, 1972; Zadoks & Schein, 1979). In this light it is very likely that the latent period of *P. lagenophorae* will increase at temperatures we did not include in our experiments. The latent period of *P. lagenophorae* has been previously reported to be approximately 10 days at 20°C (Paul & Ayres, 1984; Wyss, 1997). In the present study, the latent period was 11.3 days on average at that temperature. The slightly longer latent period recorded in this study is probably due to the lower night temperature used. The latent period of *P. lagenophorae* is, compared to *Puccinia hordei* on barley (Simkin & Wheeler, 1974), *Puccinia arachidis* on groundnut (Wadia & Butler, 1994) and *Melampsora lini* on *Linum marginale* (Burdon & Elmqvist, 1996), moderately sensitive to temperature.

Production of aeciospores gradually increased to a maximum followed by a gradual decrease with time in all treatments. This pattern has been observed in several other fungal species like *P. recondita* on wheat (Mehta and Zadoks, 1970; Eyal & Peterson, 1967), and *Pyricularia oryzae* on rice (Kato & Kozaka, 1974). The gamma distribution fitted the sporulation curves measured reasonably well, though the fit was worse for low temperature (10°C). The gamma distribution underestimated the maximum aeciospore production per plant per day in all treatments, which is due to the logarithmic transformation used to enable the use of linear-least-squares as a fitting procedure. However, the aeciospore production was well estimated by the gamma distribution.

The effect of temperature on the sporulation curves of *P. lagenophorae* was similar to, but less extreme compared to the sporulation curves of *Pyricularia oryzae* on rice (Kato & Kozaka, 1974; Van den Bosch *et al.*, 1988b). An increase in temperature during the day resulted in an increased relative spore production at the beginning of the infectious period. This shape of the sporulation curve is reflected in the quotient of the mean, μ, and standard deviation, ν, of the time required to produce a randomly selected spore after the latent period. The value of ν/μ of *P. lagenophorae* slightly decreased with increasing temperature.

The total aeciospore production per plant, and thus the net reproductive number R_0, increased by almost 50 fold when temperature increased from 10°C to 22°C. The relationship

between temperature and net reproductive number, was affected by the low aeciospore production of plants grown at 22°C in group I. Due to this treatment the value of the calibration constant, *c*, was probably underestimated.

Application of the methods to calculate the exponential growth rate, *r*, and the velocity of focus expansion *c'*, as described, made it possible to determine to which extent the effect of temperature on epidemiological parameters of individual lesions contribute to effects at the population level. Van den Bosch *et al* (1988b, 1990a) demonstrated that a decreased latent period *p*, as well as an increased standard deviation of the infectious period, ν at a fixed mean μ, a decreased μ at a fixed ν and/or an increased net reproductive number produces an increased velocity of focus expansion. The relationship between temperature and velocity of focus expansion *c'* of *P. lagenophorae* appears to be mainly caused by the effect of temperature on latent period and on the net reproductive number. Both the relative growth rate and the velocity of focus expansion were affected to the same degree by temperature, suggesting that the disease gradient (i.e., the decline in infections over distance (Campbell & Madden, 1990)) is not affected by temperature as might be expected.

Only few estimates of velocity of focus expansion are presented in literature. Zadoks & Van den Bosch (1994) presented a list with some empirical records for focal epidemics. Downy mildew on spinach, caused by *Peronospora farinosa*, can be indicated as a slow expanding disease, spreading 2.3 cm day^{-1} (Van den Bosch *et al.*, 1988c). On the other hand, the rust fungus *Puccinia coronata* on oats, spreading with a velocity of ~50 cm day^{-1}, can be indicated as a fast expanding disease (Berger & Luke, 1979). Stripe rust on wheat, caused by *Puccinia striiformis*, spreads with a velocity of 8 cm day^{-1} (Van den Bosch *et al.*, 1988c), which is comparable with the spread of *Uromyces fabae* on broad bean, *Uromyces appendiculatus* on French bean and *Puccinia recondita* on wheat (Zadoks & Van den Bosch, 1994). However, Buiel *et al.* (1989) reported a velocity of 3 cm day^{-1} for *Puccinia striiformis*. She explained this low velocity compared to the findings of Van den Bosch by unfavourable weather conditions. Our findings support this explanation.

Estimates of velocity of focus expansion of *P. lagenophorae* are available in literature. An estimate for the distribution kernel, σ= 28 cm, was obtained from field data (Frantzen & Van den Bosch, 2000). Including this value in equation (10) results in an estimated velocity between 3.5 cm day^{-1} at 10°C to 8.0 cm day^{-1} at 22°C. These results suggest that *P. lagenophorae* is a rather slow expanding fungus, which compared to the fungi presented by Zadoks & Van den Bosch (1994). However further field studies on velocity of focus expansion of *P. lagenophorae* should be executed to validate these data.

The models used in our study have been extensively verified and have been accepted in epidemiology (Levin, 1989; Zadoks & Van den Bosch, 1994). Nevertheless, field experiments are still needed to test our conclusions concerning the effect of temperature on exponential growth rate, *r*, and velocity of focus expansion *c*, by practical experience.

Our study demonstrated that temperature affects the velocity of focus expansion and exponential growth rate. These resulted in a positive relationship between temperature and both velocity of focus expansion and relative growth rate of *P. lagenophorae* on a population of *S. vulgaris* in the temperature range between 10 and 22°C. This effect was mainly caused by the effect of post-inoculation temperature on both latent period and net reproductive number. Certainly, the build up of an epidemic is affected by more than temperature alone. Aeciospores of *P. lagenophorae* need high humidity to germinate and dry periods will reduce the epidemic development. Effects of these and other factors, as plant line, plant stage and plant density on epidemics of *P. lagenophorae* are hardly known and should be subject of further research.

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Chapter 3

Estimated effects of quantitative resistance on focal expansion of *Puccinia lagenophorae.*

(Submitted to Plant Pathology)

Susceptibility of six plant lines of *Senecio vulgaris* towards the rust fungus *Puccinia lagenophorae* was determined. The most susceptible and the most resistant plant lines were pNLd and pUK, collected in the Netherlands and the UK, respectively. Latent period *p*, and aeciospore production *I(t)*, were determined for two plant stages of each of these two plant lines grown under two day-night temperature regimes (22-8°C and 22-22°C). Aeciospore production was described by a logarithmic transformation of the gamma density. The mean time to produce an aeciospore, μ, and its standard deviation ν were calculated using the parameters describing the gamma function. Velocity of focus expansion *c*, was calculated using estimates of the parameters *p*, μ and ν. Plant line and temperature affected μ and ν, while *p* was affected differently by plant line for each temperature. Calculated velocity of focus expansion was significantly affected by plant line only and was highest on the most susceptible plant line pNLd. The results suggest a difference of 10 m^2 in focus size between the most susceptible and most resistant plant line after 40 days of expansion of *P. lagenophorae* from an inoculum source.

INTRODUCTION

Senecio vulgaris (Asteraceae), common groundsel, is a self-compatible and strongly self-pollinating annual plant species originating from southern Europe (Kadereit, 1984). Plants of this species may cause problems in agriculture as yield reduction due to competition with *S. vulgaris* has been demonstrated for lettuce (Paul & Ayres, 1987a) and tomatoes (Qasem & Hill, 1994).

The rust fungus *Puccinia lagenophorae* (Basidiomycetes: Uredinales) infects *S. vulgaris* and it is known to reduce the competitive ability of this weed towards other wild species (Paul, 1989; Paul & Ayres, 1990) and crops (Paul & Ayres, 1987a; Frantzen, 2000). The rust fungus has, therefore, been proposed as a biological control agent of the weed (Müller-Schärer & Frantzen, 1996; Frantzen & Hatcher, 1997).

The weed-pathosystem *Senecio vulgaris* - *Puccinia lagenophorae* is also used to develop the system management approach of biological weed control (Müller-Schärer & Frantzen, 1996). The aim of this approach is to shift the competitive balance between weed and crop in favour of the crop by stimulating epidemics of a pathogen that reduces the competitive ability of the target weed. These epidemics should spread as fast as possible in order to obtain an effective reduction in competitive ability of the target weed (see e.g. Frantzen, 2000). Frantzen & Müller-Schärer (1998) proposed a theory to predict the impact of disease epidemics on weed-crop interactions. This theory assumed focal expansion of the disease (*P. lagenophorae*) over a host population (*S. vulgaris*).

The effect of temperature on epidemic development of *P. lagenophorae* in a population of *S. vulgaris* was quantified in Chapter 2. The focus was assumed to be rotationally symmetric. The radius of the area covered by a pathogen should increase, under constant conditions, with a constant and predictable velocity, *c*. This assumption probably results in an underestimation of *c* (Frantzen & Van den Bosch, 2000), but enables the use of an analytical model (Van den Bosch *et al.*, 1988b; 1988c) to calculate *c*. An advantage of this model is that effects of biotic and abiotic factors on the model parameters may be determined using individual plants in laboratory experiments at a relatively small scale. The

effect of daily temperature on individual epidemiological parameters was determined in the laboratory before (Chapter 2). Latent period and sporulation period decreased with increasing temperature whereas aeciospore production increased. As a result, the velocity of focus expansion increased with increasing temperature.

The aim of the present study was to quantify the effects of quantitative and race non-specific resistance and ontogenetic resistance of *S. vulgaris* (Wyss & Müller-Schärer, 1999) on *P. lagenophorae* epidemics. The effects were quantified in a similar way as described in Chapter 2. The effect of temperature was included in the present study because interactions between environment and plant lines with respect to quantitative resistance are likely to occur (Jenns & Leonard, 1985; Shaw, 1990; Figueroa *et al*., 1995; Carisse & Peyrachon, 1999).

MATERIALS AND METHODS

Plant and fungus

Full maternal sibship plant lines of *S. vulgaris* were used from Unterehrendingen, Switzerland (referred to as pCHu), from Leiden, the Netherlands (pNLd), Lancaster, United Kingdom (pUK), Wallis, Switzerland (pCHw), Greifswald, Germany (pD) and from Lienden, the Netherlands (pNLl). The plant lines were cultured for four generations to eliminate potential maternal effects as described by Wyss (1997). The plants used in the experiments described below were of the fourth generation. Seeds of all plant lines were collected from plants grown in a growth chamber at the University of Fribourg (Switzerland). The Swiss rust strain (rELS) was used. This strain had its origin in the same plant population as used to select plant line pCHu (Wyss, 1997). The rust strain was obtained from a single-aeciospore culture and maintained on host plants of line pCHu.

Pilot experiment

A pilot experiment was conducted to select the most susceptible and most resistant plant line out of the six available plant lines. Seeds of plant lines pCHu, pNLd, pUK, pCHw, pD and pNLl germinated in shallow trays filled with nutrient amended peat (Floragard TKS 2), placed in an incubator with a day-night cycle of 16 hours light and 8 hours dark, a day temperature of 22°C and a night temperature of 12°C, and a relative humidity fluctuating between 70 and 80%. Seedlings at the stage of the first true leaf were transplanted to 9-cm diameter pots and returned to the incubator. One seedling was transplanted to each pot. Ten plants of each plant line were inoculated with *P. lagenophorae* aeciospores when 3-4 true leaves had developed. Aeciospores of rust line rELS were evenly distributed over the plants using a settling tower. Plants were covered with plastic to keep a high humidity and were returned to the incubator immediately after inoculation. Plastic was removed 15 hours after inoculation.

Susceptibility of the six plant lines was assessed by determining the latent period and disease severity. Every plant was checked on open aecia daily. Latent period was defined as number of days between inoculation and presence of a first open aecium. Disease severity was defined as fraction of leaf area occupied by *P. lagenophorae* mycelium as determined by image analysis 13 days after inoculation. A leaf with open aecia was placed on a light source and covered with a glass plate. A black and white CCD-Video camera Ikegamy ICD-46E (Ikegamy Tsuskinki CO. Ltd) filmed the white light penetrating the leaf. Less light penetrated leaf parts with mycelium. The image information was transmitted into the computer program Scion NIH Image (Version 1.57). Leaf area and leaf area infected were determined.

Estimation of epidemiological parameters

Data collection

Seeds of the most susceptible and resistant plant line, pNLd and pUK respectively (see results pilot experiment), were sown in 5*5*5 cm peat pots filled with nutrient amended peat (Floragard TKS 2) and five seeds were sown per pot. The pots were placed in an incubator

with a day-night cycle of 16 hours light and 8 hours dark, a day temperature of 22°C and a night temperature of 12°C, and a relative humidity fluctuating between 70 and 80%. Twenty plants with two and four true leaves, respectively, were selected of each plant line 21 days after sowing. Plant stand was thinned to one plant per pot. Plants were inoculated with *P. lagenophorae* aeciospores and incubated as described above. Ten plants per plant stage and plant line were placed in an incubator with a day temperature of 22°C and a night temperature of 8°C. The remaining 10 plants were placed in an incubator with a constant temperature of 22°C. Both incubators had a day-night cycle of 16 hours light and 8 hours dark and a relative humidity fluctuating between 70 and 80%. Plants within peat pots were transplanted to 9-cm pots filled with nutrient amended peat (Floragard TKS 2) at 8 days after inoculation.

Plants were divided in two groups of five plants per plant line, plant stage and temperature of incubator. Latent period *p* was determined for each group of five plants as described above. Aeciospore production was determined by collecting aeciospores from each group of five plants every third day after the end of the latent period. Aluminium foil (9*9 cm) was placed below the first leaf pair in order to catch aeciospores dropped from leaves. Aeciospores were brushed from the leaves and the foil with a paintbrush and collected in a snap-cap bottle. The number of aeciospores collected was assessed using a haemacytometer. Demineralised water (5 ml) and two drops of Tween 20 were added to each bottle. The bottles were shaken until all aeciospores had been suspended in the solution. An aliquot (0.064 μl) was placed in a haemacytometer and all aeciospores present were counted. Ten aliquots were sampled from each snapcap bottle and the average was calculated. Aeciospore collection was stopped 36 days after the latent period.

Data analysis

The aeciospore production *I(t)*, was described for each group of five plants using a logarithmic transformation of the gamma density (Chapter 2):

$$\ln(I_t) = \alpha + \delta_1(\ln(t)) + \delta_2 t \qquad (1)$$

The mean time μ required for the production of a randomly selected spore after the latent period equals:

$$\mu = (\delta_1 + 1) / -\delta_2 \qquad (2)$$

The standard deviation ν, of μ equals:

$$\nu = \sqrt{(\delta_1 + 1) / -\delta_2^{\,2}} \qquad (3)$$

The shape of the spore production curve is expressed by the coefficient of variation, *CV*:

$$CV = \nu / \mu \qquad (4)$$

The velocity of focus expansion *c*, expressed in centimetre per day, was calculated for each group of five plants using estimates of the parameters *p*, μ and ν, and equation (A.1) from Van den Bosch *et al*. (1988b). The calculation also required an estimate of the standard deviation of the spatial distribution of daughter lesions around a mother lesion, σ, and the net reproductive number R_0. The net reproductive number is defined as the number of daughter lesions per mother lesion in an otherwise uninfected field. These parameters were not determined in the present experiment, but estimates of σ = 28cm and R_0= 383 were determined in another study (Frantzen & Van den Bosch, 2000).

Effects of the factors plant line, plant stage at inoculation and temperature on *p*, total aeciospore production $I_{(tot)}$, μ, ν, *CV*, and *c,* were tested on significance by means of analysis of variance (ANOVA) where all factors had two categories.

RESULTS

Pilot experiment

A significant (ANOVA, P<0.001) effect of plant line on latent period was detected. The latent period of *P. lagenophorae* was shortest on plants of line pNLd and longest on plants of line pUK (Table 1). Plant line also had a significant (ANOVA on arcsin transformed data, P<0.001) effect on severity. Fraction leaf area infected was largest on plants of line pNLd and smallest on plants of line pUK (Table 1). Plant line pNLd was thus the most susceptible of the six plant lines towards rust strain rELS and plant line pUK most resistant based on these results. Epidemiological parameters were, therefore, estimated using plants of these two plant lines (see below).

Table 1. Latent period and disease severity of *P. lagenophorae*, strain rELS, on six plant lines of *S. vulgaris*, grown at a temperature of 22°C during the day and 12°C during the night.

Plant line	Latent period	Disease severity
pCHu	11.0 a	0.28 a
pNLd	10.1 b	0.48 b
pUK	11.1 a	0.18 a
pCHw	10.6 ab	0.29 a
pD	10.5 ab	0.36 ab
pNLl	10.2 b	0.30 ab

Entries are mean values of 10 plants. Values in columns carrying different letters are significantly (P<0.05; Scheffé's test) different.

Estimation of epidemiological parameters

The latent period was longer at 8°C than at 22°C (Table 2), but this temperature effect depended on plant line (Table 3). The difference in latent period between line pNLd and line pUK was larger at a night temperature of 8°C than at a night temperature of 22°C. Overall, no significant effect of plant line on latent period, however, was detected, i.e. latent period was on average 11.2 days on plants of line pUK and 11.0 days on pNLd.

Several plants died during the period of aeciospore collection. Mortality was higher for plants grown at 22°C-22°C than for plants grown at 22°C-8°C. A relatively large number of plants of line pNLd died at both night temperatures.

A significant (ANOVA, P<0.001) effect of plant stage and a significant (ANOVA, P<0.01) effect of plant line on total aeciospore production were detected (Table 2, Table 3). Total aeciospore production was higher on plants inoculated at the four-leaf stage than on plants inoculated at the two-leaf stage. More aeciospores were produced on plants of plant line pUK than on plants of plant line pNLd.

Table 2. Latent period (p) in days, standard error of latent period (in brackets) and total aeciospore spore production (I_{tot}) of *P. lagenophorae*, strain rELS, on two plant stages of the Dutch (pNLd) and British (pUK) plant lines of *S. vulgaris* at a temperature of 22°C during the day and 22°C, or 8°C, during the night.

			22°C-22°C		22°C-8°C	
Line	Stage (leaves)	Replicate[a]	p	$I_{tot}(x10^5)$	p	$I_{tot}(x10^5)$
pNLd	2	1	10.6 (0.3)	1.99	12.0 (0.3)	3.53
pNLd	2	2	10.2 (0.2)	2.13	11.8 (0.2)	2.63
pNLd	4	1	10.0 (0.0)	8.96	11.6 (0.2)	6.99
pNLd	4	2	10.2 (0.2)	11.53	11.8 (0.2)	10.44
pUK	2	1	10.0 (0.0)	3.81	12.2 (0.2)	6.11
pUK	2	2	10.2 (0.2)	4.59	12.2 (0.2)	9.26
pUK	4	1	10.0 (0.0)	21.13	12.4 (0.3)	23.46
pUK	4	2	10.2 (0.2)	12.67	12.2 (0.2)	18.76

[a] One replicate is a group of 5 plants.

Table 3. Effects of plant line, plant stage, temperature and their interactions on latent period *p*, the mean time to aeciospore production μ, the standard deviation of the aeciospore production ν, and the velocity of focus expansion *c*, of *P. lagenophorae*, strain rELS.

		p	μ	ν	*c*
Factor and interaction	df	F^a	F^a	F^a	F^a
Line	1	3.6^{ns}	26.820^{**}	9.660^{*}	27.252^{**}
Stage	1	1.6^{ns}	1.500^{ns}	0.852^{ns}	0.009^{ns}
Temp	1	547.6^{***}	20.089^{**}	20.037^{**}	0.009^{ns}
Line * Stage	1	3.6^{ns}	1.140^{ns}	0.000^{ns}	0.441^{ns}
Line * Temp	1	14.4^{**}	1.352^{ns}	0.669^{ns}	0.225^{ns}
Stage * Temp	1	0.4^{ns}	0.040^{ns}	0.037^{ns}	0.441^{ns}
Line * Stage * Temp	1	0.0^{ns}	0.932^{ns}	0.799^{ns}	0.081^{ns}
Error	8				

[a] ns, not significant; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$

Table 4. Estimates and standard error (in parentheses) of parameters of a gamma function[a] fitted to the data of aeciospore production of *P. lagenophorae*, strain rELS on two plant stages of the Dutch (pNLd) and British (pUK) plant lines of *S. vulgaris* at a temperature of 22°C during the day and 22°C or 8°C during the night.

			22°C-22°C			22°C-8°C		
Line	Stage (leaves)	Replicate[b]	δ_1	δ_2	R^2	δ_1	δ_2	R^2
pNLd	2	1	0.84 (1.02)	-0.21 (0.07)	0.24	2.20 (0.77)	-0.26 (0.07)	0.78
pNLd	2	2	7.19 (1.96)	-0.75 (0.21)	0.77	0.49 (0.44)	-0.09 (0.03)	0.80
pNLd	4	1	4.69 (0.89)	-0.66 (0.10)	0.94	1.36 (0.24)	-0.17 (0.02)	0.97
pNLd	4	2	6.86 (1.23)	-0.98 (0.14)	0.95	1.47 (0.41)	-0.14 (0.03)	0.80
pUK	2	1	2.13 (0.79)	-0.20 (0.06)	0.66	1.50 (0.36)	-0.14 (0.03)	0.85
pUK	2	2	3.65 (0.81)	-0.27 (0.06)	0.74	1.13 (0.42)	-0.09 (0.03)	0.50
pUK	4	1	2.24 (0.51)	-0.22 (0.04)	0.87	1.27 (0.41)	-0.13 (0.03)	0.79
pUK	4	2	1.74 (0.95)	-0.18 (0.07)	0.58	1.38 (0.44)	-0.14 (0.03)	0.77

[a] $\ln(I_t) = \alpha + \delta_1(\ln(t)) + \delta_2 t$, in which I_t is spore production at time *t*, α is a constant, δ_1 and δ_2 are constants of the gamma density and *t* is the time in days after the latent period.
[b] One replicate is a group of 5 plants.

The aeciospore production curves could be fitted by the gamma distribution (Table 4). Some estimates had, however, large standard errors. Especially the spore production curve of one group of plants of line pNLd grown at 22°C and with two true leaves at inoculation was not described well by the gamma distribution. A significant (ANOVA, $P<0.05$) effect of temperature on the coefficient of variation, ν/μ, of spore production curves was detected. This coefficient equalled on average 0.67 and 0.51 on plants grown at 8°C and 22°C, respectively. As a result, sporulation curves at 8°C were flatter than at 22°C. Plant line and temperature significantly affected the parameters μ and ν describing the mean time to producing a spore and its standard deviation, respectively (Table 3). The mean time to produce a spore was on average longer on plants of line pUK than of line pNLd, *i.e.* 17.5 days and 12.0 days, respectively. The standard deviation of this time was higher on plants of pUK than of pNLd, *i.e.* 10.6 and 7.1 days. The contrary effect of μ and ν on the calculated velocity *c* resulted in a significantly slower radial spread on plants of pUK, 8.3 cm day^{-1}, than of pNLd, 9.3 cm day^{-1} (Table 3, Figure 1).

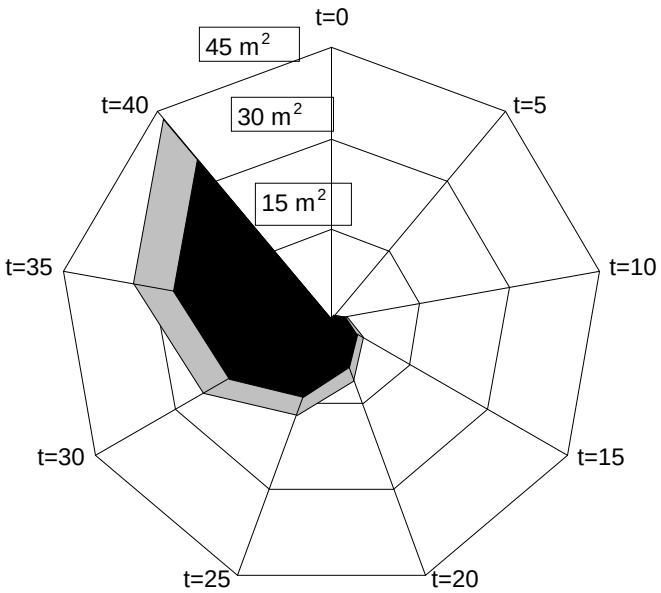

Figure 1. Focus expansion in space (in m^2) and time (in days) of *P. lagenophorae* on plant lines pNLd (□) and pUK (■).

DISCUSSION

The effect of plant line on mean time μ and standard deviation ν of the sporulation period resulted in a plant line effect on velocity of focus expansion. The shorter sporulation period on plant line pNLd compared to plant line pUK has neither been compensated for by a difference in latent period nor by a difference in the form of the sporulation curve. The estimated values of velocity of focus expansion of *P. lagenophorae* in a population of *S. vulgaris* were in the same order of magnitude as a general estimation presented by Frantzen *et al* (2001).

The present study suggested a clear effect of plant line on epidemic development of *P. lagenophorae* on *S. vulgaris*. Epidemics of *P. lagenophorae*, thus should cover and control a population of plant line pNLd faster than a population of plant line pUK .

Latent periods on plant lines pNLd and pUK were differently affected by temperature in the present study. Such interactions have been reported before in literature (Scherm & van Bruggen, 1994). Quantitative resistance has often been revealed in a plant line or cultivar

effect on latent period (Broers, 1989; Chongo & Bernier, 1999; Lehman & Shaner, 1996; Parlevliet, 1975; Wilson & Shaner, 1989). Quantitative resistance in plant lines of *S. vulgaris* towards *P. lagenophorae* was, however, more clearly revealed in disease severity and aeciospore production than in latent period. Severity was largest on plant line pNLd and aeciospore production was largest on pUK. Spore production does not necessarily correlate with severity (See Leonard, 1988; Hess & Shaner, 1987). Moreover, severity has been defined here as fraction of leaf area infected and was thus independent of leaf size. Aeciospore production depended on the actual amount of aecia and thus on leaf size too. Severity is a good indicator for infection level on a plant, aeciospore production for reproductive ability.

Contrary to the findings in Chapter 2, the effect of night temperature on the parameters of the sporulation period did not result in a temperature effect on velocity of focus expansion. Velocity increases with an increasing coefficient of variation, ν/μ, at a constant scaled latent period p/μ (Van den Bosch *et al*, 1988b; 1990a). Apparently the stimulation of the epidemic development, caused by the increasing coefficient of variation, was compensated for by the inhibition, caused by the increasing scaled latent period at increasing temperature (see Figure 4 in Van den Bosch *et al*, 1988b). As a result, the velocity of focus expansion *c* was not affected by this particular night temperature in the present study.

The gamma distribution fitted the sporulation curves reasonably well. A logarithmic transformation was, however, used to enable the use of linear-least-squares as a fitting procedure causing an underestimation of the maximum aeciospore production per plant per day (data not presented). Production of aeciospores gradually increased to a maximum followed by a gradual decrease with time on both plant lines. This pattern has been observed for *P. lagenophorae* on *S. vulgaris* before (Chapter 2) and for several other fungal species (*e.g.* Eyal & Peterson, 1967; Mehta and Zadoks, 1970; Kato & Kozaka, 1974). In general, aeciospore production was rather low compared to earlier studies (Chapter 2). This low aeciospore production was mainly explained by the early mortality of plants during the experiment. The cause of this mortality is not known, and plant line pNLd appeared to be more affected by it than plant line pUK. As a result of this early mortality, the sporulation period, expressed in mean time of aeciospore production μ and standard deviation of spore production ν, was reduced for pNLd.

The model used to analyse the effects of quantitative resistance on *P. lagenophorae* epidemics incorporated the contact distribution, the net reproductive number and the time kernel (latent period and infectious period). The contact distribution is linearly related to the velocity of focus expansion (Zadoks & Van den Bosch, 1994) and small changes of the contact distribution may have relatively large effects on the velocity. However, the quantification of the contact distribution is, in general troublesome (Frantzen & Van den Bosch, 2000) and precludes detection of the relatively subtle effects of quantitative resistance, *e.g.* by altered morphology of *S. vulgaris*, on the contact distribution. The use of an unaffected contact distribution seemed therefore reliable in the present study. The velocity of focal expansion is logarithmically related to the net reproductive number R_0, (Zadoks & Van den Bosch, 1994). Minor effects of quantitative resistance on R_0, *e.g.* by altering total spore production by a lesion and the infectivity of the spores released, will only have little effect on the velocity. The time kernel is easy to determine, like done in the present study and it may be concluded that determining the effects of quantitative resistance of *S. vulgaris* on the time kernel is most realistic to predict effects on epidemic spread.

The velocity of focus expansion has been calculated in the present study assuming focal expansion of *P. lagenophorae* epidemics in populations of *S. vulgaris*. Focal epidemics may spread rotational symmetrically as a travelling wave with a constant velocity *c*. This assumption probably resulted in an underestimation of epidemic development in practice. Epidemics of *P. lagenophorae* appear to spread as so-called dispersive waves with an increasing velocity of focus expansion (Frantzen & Van den Bosch, 2000). The spread of a dispersive wave in space, however, is not uniform and time and place of infection are less predictable than for a travelling wave. The more rapid spread of *P. lagenophorae* may thus have disadvantages with respect to providing predictable results needed for a successful use

of the system management approach of biological weed control. The first two generations of an epidemic in a young population are, however, the most important using this approach for control of *S. vulgaris* because competitive ability of *S. vulgaris* is mostly reduced during this period (Frantzen, 2000; Chapter 5). Predictions of epidemic expansion using travelling or dispersive wave models do not differ that much yet during this period (Frantzen & Van den Bosch, 2000). Until better predictions of dispersive waves are possible, epidemic expansion will therefore be treated as a travelling wave.

A successful biological weed control using the system management approach depends on artificial induction of an epidemic that reduces competitive ability of a weed. In general, populations of *S. vulgaris* have to develop from almost zero in agricultural sites in spring (Leiss & Müller-Schärer, 2001b). Whereas *P. lagenophorae* overwinters in *S. vulgaris* (Frantzen & Müller-Schärer, 1999) natural inoculum sources will hardly be available in agricultural sites. Artificial introduction of the rust as discussed in Frantzen and Müller-Schärer (1998) is then necessary. The present study demonstrated that the introduction of a more aggressive rust strain in a susceptible *S. vulgaris* population increased development of the epidemic. Small laboratory experiments, as described in the present study, could be used to estimate epidemic development of a rust strain on a host population. With this knowledge, the introduction of inoculum to induce an epidemic that shifts competitive balance away from *S. vulgaris* can further be optimised.

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Chapter 4

Does a disease epidemic shift the competitive balance between weeds?

The spatial and temporal dynamics of an induced epidemic of the rust fungus *Puccinia lagenophorae* on a population of its host *Senecio vulgaris* grown in an 8:1 mixture with *Capsella bursa-pastoris* were quantified in a field experiment. The effect of this epidemic on competitive balance between both plant species was quantified at the end of the experiment. A host plant with sporulating aecia was planted in the centre of the experimental plot. Open aecia on *S. vulgaris* were counted to quantify epidemic spread at regular intervals. A non-random aggregated spatial pattern of open aecia was observed during the whole experiment. A clear focus was formed in the first generation. The spore dispersal gradient (or infection progress curve) was described well by the power law equation at 14 and 28 days after introduction of the inoculum source (a.i.), but not at 32 days a.i. The second generation of the rust epidemic had started between 28 and 32 days a.i., flattening the curve. An infection progress curve described by the power law equation that flattens in time suggests that the epidemic spreads like a dispersive wave with an increasing velocity. Spread of later generations of this type of epidemics is more difficult to predict, than early generations that do not differ much from epidemics that spread like a travelling wave Competitiveness of *C. bursa-pastoris* towards *S. vulgaris* was quantified using total pod production at the end of the experiment. Competitive balance between the two weeds was not changed in favour of *C. bursa-pastoris* by rust infection. The infection level obtained appeared to be too low to reduce competitive ability of the host at a detectable level.

INTRODUCTION

Müller-Schärer & Frantzen (1996) presented the system management approach of biological weed control as alternative to the classical and inundative ones. This approach is based on a shift in competitive balance between crop and weed in favour of the crop by inducing an epidemic of a specific weed pathogen. The weeds *Capsella bursa-pastoris* (Brassicaceae) and *Senecio vulgaris* (Asteraceae) were chosen as a study model. The host specific rust fungus *Puccinia lagenophorae* (Basidiomycetes: Uredinales), infecting the latter weed species, was chosen as plant pathogen.

Both weeds are members of the community of waste ground, arable and garden weeds in Central Europe (Aksoy *et al*., 1998; Kadereit, 1984). *C. bursa-pastoris*, shepherd's purse, and *S. vulgaris*, groundsel, are self-compatible and strongly self-pollinating annual plant species. *C. bursa-pastoris* outcompetes healthy *S. vulgaris* under nutrient-poor conditions. Under nutrient-rich conditions, *S. vulgaris* has a competitive advantage over *C. bursa-pastoris*. Infection of *S. vulgaris* with *P. lagenophorae* eliminates this advantage again (Paul & Ayres, 1990).

P. lagenophorae overwinters in groundsel plants, infected in autumn, but equally causes high winter mortality of its host (Paul & Ayres, 1986c; Frantzen & Müller-Schärer, 1999). As a result, only few inoculum sources are available in spring and it takes until summer before epidemics develop (Leiss & Müller-Schärer, 2001b). Early artificial introduction of inoculum sources overcomes this delay in epidemic development. (Frantzen & Müller-Schärer, 1998). Epidemics starting from these inoculum sources may create disease foci, which expand by reproduction and dispersal of the pathogen (Zadoks & Schein, 1979). Two models (amongst others) have been introduced to describe epidemic development: the travelling and dispersive wave. Epidemics of *P. lagenophorae* have often been assumed to be focal and expanding at a constant velocity as a so-called travelling wave (Frantzen &

Müller-Schärer, 1998; Chapter 2; Chapter 3). Field studies suggested, however, an epidemic expanding at increasing velocity as a so-called dispersive wave (Frantzen, 2000; Frantzen & Van den Bosch, 2000; Frantzen *et al*, 2001). Plant-disease dispersal gradients can be used to model epidemic expansion by fitting contact distributions to data obtained in field experiments (Kot *et al*., 1996). The choice between the two models is strongly influenced by the dispersal gradient best fitted.

The epidemiology of *P. lagenophorae* on a population of *S. vulgaris* and its effect on the competitive balance between *S. vulgaris* and *C. bursa-pastoris* were linked in a field experiment. An epidemic was induced from the centre of a field plot. The type of epidemic spread (travelling or dispersive wave) was determined by the dispersal gradient of sori, caused by infection. Change in competitive balance within this epidemic was estimated by determining pod production of *C. bursa-pastoris*. It was hypothesised that pod production in the centre of the epidemic would be higher than at its outer border where plants would get infected later than near the inoculum source in the centre.

MATERIAL AND METHODS

Plant and fungus

Plants of *S. vulgaris* line 'pCHu' and *Capsella bursa-pastoris* line 'BGF' were used. The full maternal sibship plant line 'pCHu' originated from a plant collected in Unterehrendingen (Switzerland) during a field survey in 1993. Selection and cultivation of the groundsel lines is described by Wyss (1997). Plant line 'BGF' originated from a plant collected in the botanical garden of Fribourg (Switzerland) in 1996 (Frantzen, personal communication). The first generation of this plant line was produced from seeds of one fruit of one plant. The Swiss rust strain (rELS) was used. This strain has its origin in the same plant population as used to select plant line 'pCHu' (Wyss, 1997). The rust strain was obtained from a single-aeciospore culture and maintained on host plants of line 'pCHu'.

Plant production and experimental plots

Shallow trays were filled with nutrient amended peat (Floragard TKS 2). Seeds of *S. vulgaris* were sown and grown in a greenhouse at the Novartis Research Station in St. Aubin (Switzerland) in 1998. Seeds of *C. bursa-pastoris* were sown and germination was induced by stratification in an incubator with a regime of 11 hours without light at 4°C and 1 hour at 15°C to prevent ice formation inside the incubator, for one week. Relative humidity fluctuated between 70 and 80%. Trays with stratified seeds were moved to the greenhouse and grown under the same conditions as *S. vulgaris*.

The experiments were carried out at a lawn at the University of Fribourg (Switzerland). Two experimental plots of 8.1m by 8.1m were marked. In each plot, 13 by 13 patches of 0.3m by 0.3m were marked. The distance between the patches was 0.3m. Grass was removed from the patches and the patches were kept free of weeds during the length of the experiment. The grass around the patches was frequently mown to eliminate the competitive effects of grass.

Transplant and inoculation

Both *S. vulgaris* and *C. bursa-pastoris* were transplanted to the experimental field on 18 June 1998, 21 days after sowing. Plants of *S. vulgaris* had 2-4 true leaves and *C. bursa-pastoris* had 2 true leaves. One plant of *C. bursa-pastoris* was planted in the centre of each patch. Eight plants of *S. vulgaris* were planted in a regular pattern around *C. bursa-pastoris*. The central patches of both plots were kept free for one week. A plant with sporulating sori was transplanted to the central patch of one plot on 25 June 1998 in the evening. The second plot served as a control.

Data collection

S. vulgaris was examined daily for the presence of open aecia during 19 days after introduction of the sporulating plant (a.i.). The number of open aecia per patch was counted each third day between 14 and 34 days a.i. Number of patches with sporulating plants, number of sporulating plants per plot and number of sporulating sori per patch were determined. Plants of *C. bursa-pastoris* were collected from the inoculated and control plot 32 days a.i and 34 days a.i. respectively and pods were counted.

Spatial analysis

Spatial autocorrelation of the number of open sori per patch, i.e. the probability that the number of sori measured in a patch is similar to the number of sori measured in neighbouring patches, was quantified using the Moran statistic. Spatial autocorrelation was determined 14, 28 and 32 days after transplantation and computed as (Frantzen, 1994):

$$I = \frac{n\sum_{ij} w_{ij} z_i z_j}{W\sum_{i=1}^{n} (z_i)^2} \qquad (1)$$

and

$$z_i = x_i - \bar{x} \text{ and } z_j = x_j - \bar{x} \qquad (2)$$

in which I is the Moran statistic with a value between -1 and +1, n is the number of patches of a plot occupied by plants, w_{ij} a weight that defines two patches i and j as neighbours or not, W is the sum of the weights, x_i is the number of sori in patch i, and x_j is the number of sori in patch j. Patches were defined as neighbours by distance, in which the maximum distance $\sqrt{2}$ referred to the eight patches adjacent to a patch. Whether the I value differed significantly from zero was tested by a randomisation test (Sokal & Rohlf, 1995). Significant positive values indicate that patches with sori are aggregated and significant negative values that patches with sori avoid each other. Values not significantly different from zero indicate a random spatial pattern of patches with sori. Spatial autocorrelation of the date the first open aecium in a patch was observed and the distance of this patch from the central patch was also determined.

The functions describing the spore dispersal gradients at 14, 28 and 32 days after transplantation were estimated. The power law model (Equation 4) and the exponential model (Equation 5) were fitted to the number of open sori per patch (Frantzen & Müller-Schärer, 1998):

$$y = a \cdot x^{-b} \qquad (4)$$

and

$$y = c \cdot e^{(-d \cdot x)} \qquad (5)$$

in which a and c are constants related to the source strength, and b and d are parameters indicating the steepness of the spore-dispersal gradient. Models were fitted to the data using non-linear regression.

The association between number of sporulating sori on *S. vulgaris* and number of pods of *C. bursa-pastoris* in the same patch at 29 days a.i was determined using correlation analysis. Additionally the association was determined between the time after introduction of the inoculum source in the field until the first open aecium on *S. vulgaris* in a patch was

observed, and the number of pods produced by *C. bursa-pastoris* in that same patch. The association between pod production per plant of *C. bursa-pastoris* and distance from the central patch was determined using linear regression.

RESULTS

The inoculum plant survived 25 days in the field. Sporulation was only observed within a few days after transplant (a.i.). The first symptoms of disease were observed in the inoculated plot 9 days a.i. First sporulating aecia were observed 3 days later. The number of patches with plants bearing aecia increased to a maximum at 22 days a.i. in the inoculated plot (Figure 1). Far less patches with sporulating aecia on groundsel plants were observed in the control plot 22 days a.i. Symptoms of rust infection were observed in all patches in the inoculum plot and in 98.8% of the patches in the control plot at the end of the experiment (32 days a.i.). Number of open aecia in the control plot stayed far below the number of open aecia in the inoculated plot during the whole experiment (Figure 2). The increase of the number of open aecia flattened in the inoculum plot in the period between 19 and 25 days a.i. followed by a steep increase 32 days a.i. indicating the spread of the second generation of the pathogen.

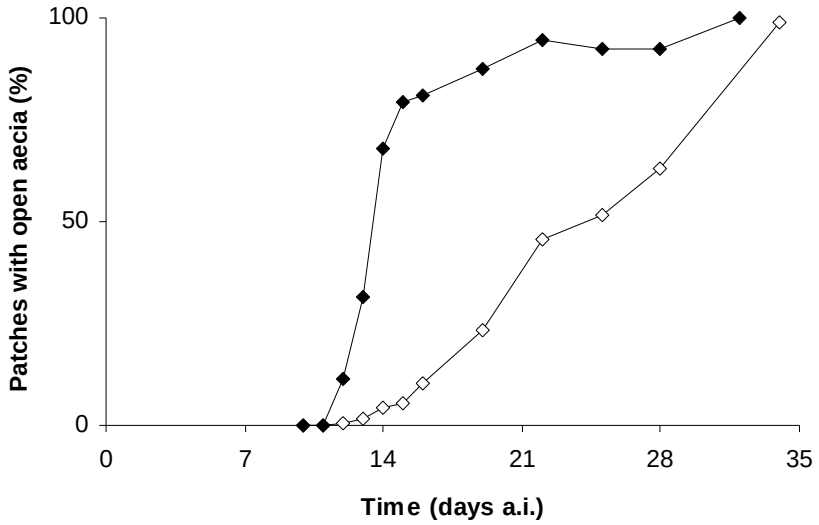

Figure 1. Patches with plants bearing open aecia in the inoculum plot (◆) and in the control plot (◇) against time after introduction of inoculum source.

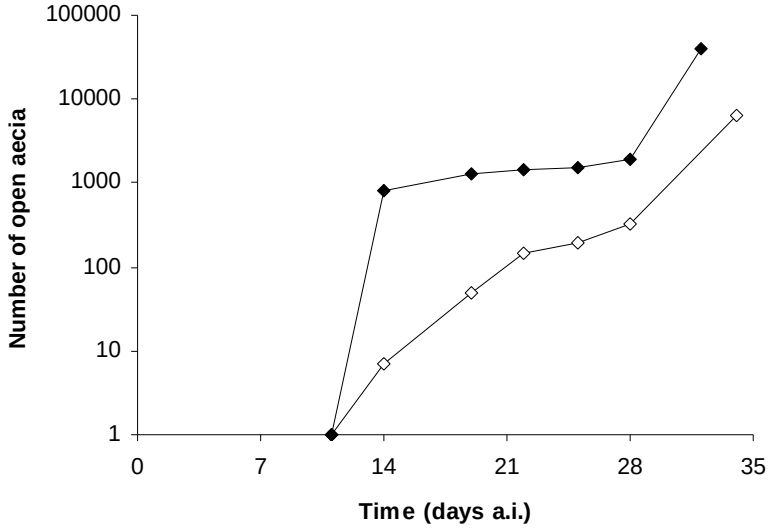

Figure 2. Number of open sori in the inoculated plot (◆) and in the control plot (◇) against time after introduction of inoculum source.

Spread of *P. lagenophorae* appeared to be focally until 28 days a.i. in the inoculated plot (Figure 3). A more general spread was observed four days later. Moran statistics indicated a non-random aggregated spatial pattern of open aecia in the inoculated plot at all three dates. The values of *I* were 0.53, 0.54 and 0.33 on 14, 28 and 32 days a.i. respectively. All values differed significantly ($P<0.001$) from zero. A random distribution of open aecia was observed until 28 days a.i. in the control plot. A non-random aggregated spatial pattern of open aecia, however, was observed in the control plot 34 days a.i. The value of *I* was 0.12 at this date, which differed significantly ($P<0.01$) from zero. Moran statistics indicated a non-random aggregated spatial pattern of the first occurrence of open aecia in a patch. The value of *I* was 0.24 and differed significantly ($P<0.001$) from zero. The infection progress curve was described well by a power law equation at 14 and 28 days a.i. in the inoculum plot (Table 1; Figure 4). The exponential model underestimated the number of open aecia at a distance of more than 1m from the inoculum source. No model described the infection progress curves well at 32 days a.i. (Table 1).

Table 1 Estimates and standard errors (in parentheses) of parameters of a power law model[*] and an exponential model[*] fitted to the data of number of aecia of *P. lagenophorae* on *S. vulgaris* plants in the inoculum plot.

Time	Power law model			Exponential model		
(d.a.i.)[x]	a^y	b^z	R^2	c^y	d^z	R^2
14	22.5 (1.0)	2.40 (0.10)	0.88	348.0 (35.0)	2.58 (0.14)	0.87
28	43.4 (1.7)	1.99 (0.08)	0.83	377.9 (35.9)	1.99 (0.12)	0.80
32	407.2 (47.1)	0.56 (0.13)	0.06	407.1 (83.2)	0.18 (0.07)	0.02

[*]see text, [x] d.a.i.= days after inoculum plant was transferred into the centre of the inoculum plot, [y] constant related to the source strength, [z] parameter indicating the steepness of the spore dispersal gradient.

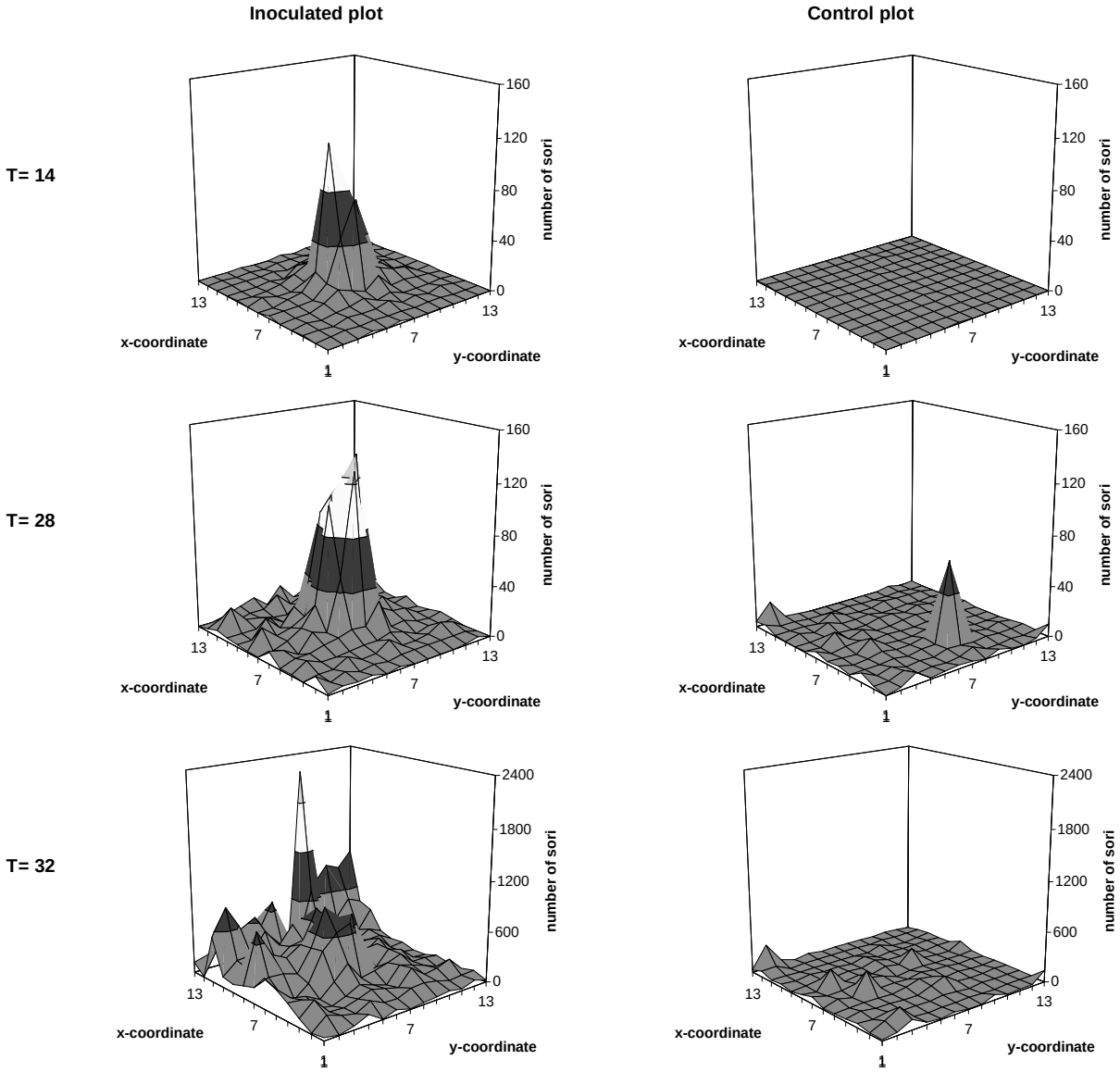

Figure 3. Spatial patterns of number of sporulating sori in the inoculated and control plot at 14, 28 and 32 days after introduction of inoculum source.

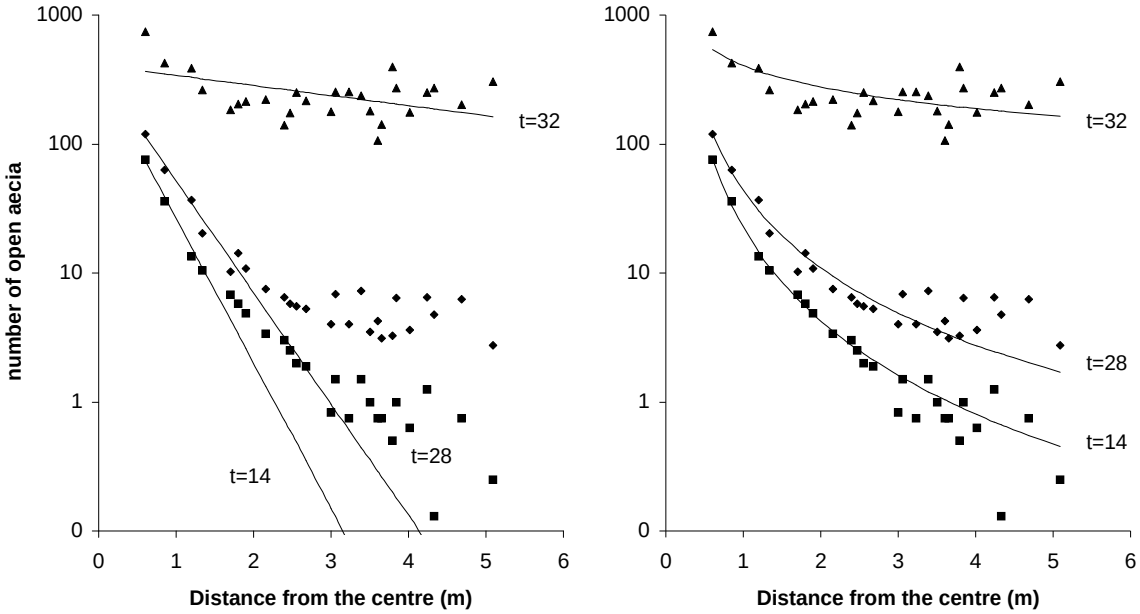

Figure 4. Curves (-) fitted to data of *P. lagenophorae* dispersal obtained at 14 days (■), 28 days (◆) and 32 days (▲) after inoculation of the plot according to the exponential (left) and power law (right) distribution. The corresponding parameter estimates and the goodness-of-fit are presented in Table 1.

Pod production of *C. bursa-pastoris* and the variation in pod production between individual plants were higher in the control plot than in the inoculated plot. Pod production per plant increased with increasing distance from the central patch in both plots. Linear regression fitted to the data resulted in low values of R^2 in the control plot (R^2= 0.062) and inoculated plot (R^2= 0.165). Number of pods produced per plant was positively correlated (0.38 in inoculated plot and 0.22 in control plot) with the date the first open aecium was observed in the same patch. Correlations were significantly (P< 0.01) different from zero in the inoculated plot and the control plot (Figure 5). No significant correlation between the number of open aecia on *S. vulgaris* in a patch and number of pods of *C. bursa-pastoris* in the same patch was demonstrated (data not presented).

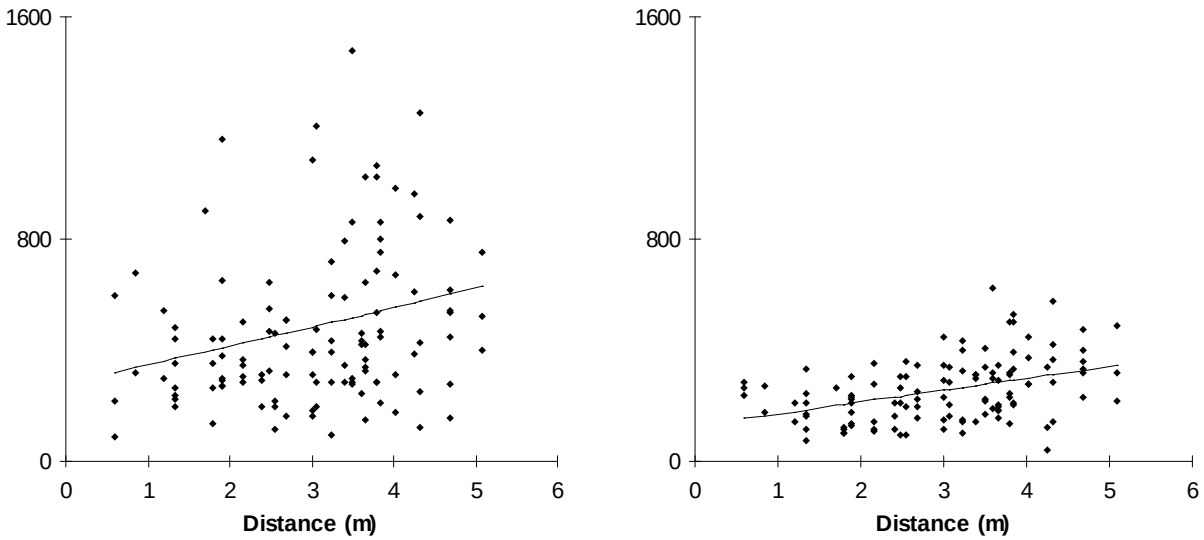

Figure 5. Linear regression (-) fitted to data of pod production per plant of *Capsella bursa-pastoris* (◆) at distance from the centre of the inoculated (left; y=68.83x+277.63) and control plot (right; y=42.83x+127.79).

DISCUSSION

Introduction of a *P. lagenophorae* inoculum source at the centre of a plot with a 1:8 mixture of *C. bursa-pastoris* and *S. vulgaris* induced an epidemic on the latter species. The spatial and temporal distribution of one generation of *P. lagenophorae* over a *S. vulgaris* population could be determined. Rust infections were also observed in the control plot, but not in an aggregated spatial pattern. Infection level in the inoculated plot increased from 0 to $\pm$ 1000 open aecia between 9 and 14 days after introduction of the source (a.i.). This level was reached in the control plot $\pm$ 16 days later after a gradual increase starting at the same day as in the inoculated plot. Artificial inoculation thus created an advance of more than one generation compared to the natural infections. The initial infections in the control plot appeared to mainly represent the natural background level and did not appear to be caused by the inoculum source of the inoculated plot. The pattern was randomly distributed and not more aggregated towards the side of the inoculated plot. Later infections in the control plot might be caused by spores released from aecia in the inoculated plot, overestimating the natural background level.

The second generation of *P. lagenophorae* already crossed the borders of the experimental plot 32 days a.i. This value equals a velocity of epidemic expansion of $\pm$ 16 cm day^{-1}, which is twice the velocity of 8 cm day^{-1} estimated in a laboratory trial (Chapter 2). This estimate was based on a net reproductive number R_0 of 580 at an average temperature of 17.33°C (see Chapter 2, Table 2), a distribution kernel σ of 28cm (Frantzen & Van den Bosch, 2000) and focal expansion as a travelling wave. The average temperature during the field trial lay just below 20°C (data not presented), only partly explaining the difference in velocity of spread between the laboratory and field data.

The dispersal gradient of the first generation of the induced rust epidemic was well fitted by the power law model. Contrary to an earlier study (Frantzen & Müller-Schärer, 1998) the exponential model fitted worse, clearly underestimating the tail of the gradient. Additionally the dispersive gradient flattened between the first and second generation. Both characteristics of the dispersive gradient suggested that the epidemic expanded over the population like a dispersive, instead of a travelling wave. Whereas the power law is not an exponentially bounded model it does not result in a constant wave of focus expansion (Shaw, 1995). An increased velocity of focus expansion over more generations is than expected. Frantzen & Van den Bosch (2000) were the first to demonstrate an epidemic of *P. lagenophorae* expanding like a dispersive wave with increasing velocity in a population of *S. vulgaris*. They monitored 2 to 3 generations of the rust. Although two generations of the present study were not enough to determine the right model to be used, the results added to the suggestion that an epidemic of *P. lagenophorae* expands like a dispersive wave in a population of *S. vulgaris*. The lower velocity of epidemic spread predicted in the laboratory compared to the field experiment therefore appeared to be mainly explained by the assumed wave type in the model used in the laboratory experiment (Chapter 2)

The development of an epidemic that spreads like a dispersive wave is more difficult to predict than a travelling wave. The way of expansion of the first one or two generations of both wave types does however not differ much yet. Clear differences can be observed between later generations. The dispersive wave not only develops with an increased velocity of expansion over time, but also with a faster expansion for lower than higher severity levels (flattening of the dispersal gradient) (Frantzen & Van den Bosch, 2000). A part of the population can thus get infected relatively fast, but disease build-up on this part will stay behind. The initially closed wave front of a dispersive wave will therefore disappear after several generations. New infection sites then become very difficult to predict.

Single and multiple inoculations of *S. vulgaris* with *P. lagenophorae* have been reported to shift the competitive balance away from *S. vulgaris* when grown in a mixture with carrots (Grace & Müller-Schärer, 2003), celeriac (Frantzen, 2000), lettuce (Paul & Ayres, 1987a), *Euphorbia peplus* (Paul, 1989) but also with *C. bursa-pastoris* (Chapter 5.; Paul & Ayres, 1990). The induced epidemic did not appear to affect the competitive balance between the two weeds in the present study. Pod production of *C. bursa-pastoris* did not increase when grown in a patch with *S. vulgaris* near the inoculum source. Surprisingly, pod

production increased with increased distance from the centre in both the inoculum and control plot. It was even higher in absence of a central inoculum source whereas it was hypothesised that the competitive ability of *S. vulgaris* towards *C. bursa-pastoris* would be decreased by rust infection. Pod production appeared to be biased by a field gradient, stronger than competition with *S. vulgaris*. The plots had been prepared just before the experiment started and some gradients in soil nutrient levels might have been caused by unequal incorporation of the soil. Another factor could have been light distribution in the field (Ballaré *e.a.*, 1997). Plants close to the border of the plots are hypothesised to have suffered less from the shadowing capacity of neighbouring plants, than plants in the centre of the plot. A random design with artificial inoculation of groundsel in patches has been used in a subsequent trial in order to exclude these gradient effects on pod production (chapter 5).

Infection of *S. vulgaris* by *P. lagenophorae* took place when plants were about 22 days old. Although the potential infectious period of the rust can be several weeks (Chapter 2 and 3), the inoculum plant already stopped sporulating after some days. Inoculations of *S. vulgaris* up to 31 days after sowing were still able to reduce pod production of *C. bursa-pastoris* surrounded by 4 or 8 host plants (Chapter 5). Plant age at inoculation is therefore not considered to explain the absence of the hypothesed effect of host infection on competitive balance in the present study. The level of disease caused by the rust epidemic appeared to be too low to seriously hamper development of *S. vulgaris*. An infection level of ± 500 sori per plant (*S. vulgaris*) reduced celeriac weight in a field trial by Frantzen (2000). Infection levels near the inoculum source were much lower in the first rust generation in the present study. At the end of the experiment (32 a.i.) mortality of *S. vulgaris* was still low (data not presented).

Frantzen (2000) and Grace & Müller-Schärer (2003) demonstrated that the use of more than one sporulating plant as inoculum source reduced competitive ability of *S. vulgaris*. A similar effect on competition under a high disease pressure was demonstrated in Chapter 5. The use of a single plant with sporulating sori as inoculum source in a field with this size appeared to be too low to induce any detectable effect on competition between the two weed species in the present study. Plant line pCHu, used in the present study, is not the most susceptible plant line known to the rust strain used (Chapter 3). Use of a more susceptible plant line could have increased the effect of the rust epidemic on competitive balance between host and non-host.

The present study demonstrated that introduction of *S. vulgaris* with sporulating aecia of *P. lagenophorae* as inoculum source induced a rust epidemic in the weed population. The results suggested that the epidemic expanded over the host population like a dispersive wave. The system management approach of biological weed control is based on induction of an epidemic with predictable focal expansion like a travelling wave (Frantzen & Müller-Schärer, 1998). Epidemic spread like both wave types is theoretically not very different yet in early generations (Frantzen & Van den Bosch, 2000). Although not confirmed yet, these first generations appear to be important to shift the competitive balance between host and non-host. The disease pressure on infected host plants did not affect the competitive balance between the host and *C. bursa-pastoris* in the present study. For a successful use of the system management approach the amount of inoculum sources needed to induce an epidemic that causes a disease pressure high enough to suppress *S. vulgaris*. should be determined. This amount will depend on both biotic (*e.g.* plant line, rust strain) and abiotic factors (*e.g.* temperature, humidity, plant density, plot size) affecting epidemiology of *P. lagenophorae* and its effect on competitive ability of *S. vulgaris*. The present study demonstrated that although underestimating the velocity of focal expansion the model used in chapter 2 and 3 can be used to predict the development of early generations of the epidemic. Further research should aim at the optimal inoculation time, rust strain-plant line combination, and disease pressure needed to reduce competitive ability of *S. vulgaris* towards competing plants under field conditions.

Chapter 5

Pathogen impact: the importance of infection time on plant growth and interspecific competition.

(Submitted to Basic and Applied Ecology)

Plant pathogens are assumed to be important factors determining the species composition of plant communities. Their impact on intraspecific competition has been well studied but little is known about the impact of time of infection on interspecific competition.

The effect of the time the weed *Senecio vulgaris* was infected with the rust fungus *Puccinia lagenophorae* on its growth and competitive ability towards *Capsella bursa-pastoris* was determined in a field study. Four or eight plants of *S. vulgaris* were grown under fertilised and non-fertilised conditions, around one plant of *C. bursa-pastoris*. The first plant species was inoculated once with *P. lagenophorae* 3, 9 or 16 days after transplant or was not inoculated (control). Time of infection with *P. lagenophorae* had a clear effect on growth and development of *S. vulgaris*. This was translated into a small shift in competitive balance for early infections only. A smaller reduction in pod production was observed when *C. bursa-pastoris* was grown between early-inoculated *S. vulgaris* as compared to control plots. Neither fertilisation nor *S. vulgaris* density influenced this effect. The present field study showed that the effect of the rust on the performance of its host plant depended on time of infection, fertilisation and plant density. Competitive balance of the host plant towards neighbouring *C. bursa-pastoris* was equally changed by the earliest two infections only and thus depended on time of infection. A better knowledge of the factors influencing the effect of host specific pathogens on plant species interactions in natural habitats may be applied to biologically managed crop-weed interactions using naturally occurring pathogens.

INTRODUCTION

In general, an infection of a plant pathogen reduces the growth and development of the host plant. As a consequence, the species' competitive ability towards neighbouring healthy plants is often reduced. The outcome of this balance is determined by availability of resources like nutrients, space and light and is often density dependent (Begon, 1990). Direct competition for space and light between two neighbouring plants does not start before the available surface is covered and plants are large enough to withhold light from their neighbours (see e.g. reviews by Clay 1990; Alexander & Holt 1998). Therefore competition for light and space will start earlier in high density populations, with plants growing close to each other, than in low density populations. Early germination, fast growth and development, plasticity to environmental changes and fast recovery can improve a plant's initial advantage over its competitors (see *e.g.* Begon, 1990; Baumann *et al.*, 2001). Early and fast growing weeds, for example, reduce crop yield in agriculture in this way. If these weeds infest the crop later in the growing season, or when the weed's growth rate is reduced by a pathogen, it is expected to be outcompeted by the crop itself. The system management approach of biological weed control (Müller-Schärer & Frantzen, 1996) is a proposed practical use of this ability of weed pathogens. Similarly, in natural or semi-natural communities like ruderal sites or waste grounds winter annuals have a competitive advantage over newly germinated plants in spring. This balance can shift when the wintered populations start to die later in the season and a new population has to be build up from seed. These young plants have to compete with the populations of plants that germinated earlier in the season. The effect of plant pathogens on these dynamics has hardly been studied.

Several studies demonstrated a shift in the competitive balance between plant species when a pathogen specific to one species was introduced (Groves & Williams, 1975; Burdon & Chilvers, 1977; Paul & Ayres, 1987a; Paul, 1989; Paul & Ayres, 1990). Nevertheless, the differences between the effects of early and late infections on the competitive balance between a host and a non-host have only rarely been studied in detail under field conditions. An exception is published by Frantzen (2000) who studied the shift in competitive balance between *Senecio vulgaris* (Asteraceae) and celeriac caused by an epidemic of *Puccinia lagenophorae* (Basidiomycetes: Uredinales) in a cultural habitat. The competitive ability of *S. vulgaris* towards celeriac was only reduced close to the inoculum sources from which the epidemic started. The expanding rust epidemic appeared to infect hosts further away from the rust sources too late to reduce the host's competitive advantage towards celeriac.

In agricultural sites natural rust incidence levels increase strongly after *S. vulgaris* populations start to increase, but in general high disease pressure appears to be initiated too late to affect the population dynamics of the plant. In ruderal habitats rust incidence levels also appear to fail to become high enough to affect growth and development of *S. vulgaris* stages. Thus, in these habitats population dynamics of *S. vulgaris* does not appear to be determined by naturally occurring *P. lagenophorae* on short term. Additionally, younger stages of *S. vulgaris* were found to be less susceptible to *P. lagenophorae* infection, than older stages, which may further reduce the effect of the rust on population dynamics and interspecific competition (Leiss & Müller-Schärer, 2001b; Wyss & Müller-Schärer, 1999). The aim of the present study was to further investigate the effect of density and plant stage at infection with *P. lagenophorae* on the interspecific competitive ability of *S. vulgaris*.

Senecio vulgaris, groundsel, is a member of the community of waste ground, arable and garden weeds in Central Europe. It is a self-compatible, strongly self-pollinating annual plant species (Kadereit, 1984) that can produce up to three generations per year (Haldimann, Steinger & Müller-Schärer, 2003). Plants may survive winter vegetatively and start seed setting in spring. The soil seed bank of *S. vulgaris* is of minor importance due to nearly complete absence of dormancy and short seed survival in soil (Popay & Roberts, 1970; Roberts & Feast, 1972).

The autoecious rust fungus *P. lagenophorae* is the most prevalent pathogen of *S. vulgaris* in Europe (Frantzen & Hatcher, 1997; Wyss & Müller-Schärer, 1999), capable of rapid disease build-up and high levels of infection. *Puccinia lagenophorae* is understood to have first infected *S. vulgaris* in Australia and then spread to *S. vulgaris* in Europe in the early 1960s (Viennot-Bourgin, 1964). The rust fungus colonises leaves, stems and capitula of *S. vulgaris* by way of aeciospores (Wilson, Walshaw & Walker, 1965) and reduces development of the plant. Leaf area, vegetative biomass, number of capitula and reproductive biomass of infected *S. vulgaris* are reduced (Leiss & Müller-Schärer, 2001b). Infection by the rust also increases the host's vulnerability to environmental stress (Paul & Ayres, 1984; 1986a; 1986b; 1986c; 1987c). Contrary to larger plants small plants infected in autumn do therefore generally not survive winter. The rust hardly grows systemically. While infected plant parts die mostly and the rust does not reproduce during winter the surviving plants are nearly free of rust in spring and the rust population must thus start from the few plants in which its mycelium was able to survive winter (Frantzen & Müller-Schärer, 1999). Healthy *S. vulgaris* have a competitive advantage over individuals that suffered from the rust, as demonstrated by Paul & Ayres (1986d).

The non-host *Capsella bursa-pastoris*, shepherd's purse (Brassicaceae), a self-compatible and strongly self-pollinating annual, is also a member of the community of waste ground, arable and garden weeds in Central Europe (Aksoy, Dixon & Hale, 1998). The competitive balance between *C. bursa-pastoris* and *S. vulgaris* depends on availability of nutrients. *S. vulgaris* has a competitive advantage over *C. bursa-pastoris* under nutrient-rich, but not under nutrient-poor conditions. Infection of *S. vulgaris* with *P. lagenophorae* eliminates the advantage of the host over *C. bursa-pastoris* (Paul & Ayres, 1990). Here, we examine to which extend this ability of the rust depends on the time *S. vulgaris* became infected.

We carried out a field study to determine the effects of the time *S. vulgaris* gets infected by *P. lagenophorae* on the host plant's growth and development, and its competitive

ability towards *C. bursa-pastoris*. A second weed as competitor instead of a crop was used to obtain information about the effect on interspecific competition under more natural than agricultural conditions only. Groups of plants were sprayed once with a spore suspension of *P. lagenophorae* at different dates and a severe infection was established to prevent plants from outgrowing the rust infection. Plant size and reproductive capacity of *S. vulgaris* are strongly affected by nutrient availability. Moreover, populations of *S. vulgaris* in fertilised agricultural sites are generally denser than in ruderal populations. (Leiss & Müller-Schärer, 2001a). Nutrient availability also influences the negative effect of rust infections on vegetative biomass of the host (*e.g.* Paul & Ayres, 1987a; 1990). We therefore included different fertilisation treatments and plant densities and hypothesised that early rather than late rust infections of *S. vulgaris* with *P. lagenophorae* reduces the host's performance and thus also its competitive advantage towards *C. bursa-pastoris*, especially under nutrient-rich conditions.

The main questions addressed were:

i) What is the effect of time of infection of *S. vulgaris* with *P. lagenophorae* on plant growth and development of the host?

ii) Does this effect depend on fertiliser treatment and on host density?

iii) Do observed effects on *S. vulgaris* performance translate on competitive ability towards *C. bursa-pastoris*?

MATERIAL AND METHODS

Plant and fungus

Plants of *S. vulgaris* line 'pCHu' and *C. bursa-pastoris* line 'BGF' were used. The selfed *S. vulgaris* line originated from a plant collected in Unterehrendingen (Switzerland) during a field survey in 1993 (Wyss, 1997). The *C. bursa-pastoris* line originated from a plant collected in the botanical garden of Fribourg (Switzerland) in 1996 (Frantzen, personal communication). The rust fungus strain (rELS) used was collected from the same plant population as used to select plant line 'pCHu' (Wyss, 1997). The rust strain was obtained from a single-aeciospore culture and maintained on host plants of line 'pCHu'.

Plant production and experimental plots

Shallow trays were filled with nutrient amended peat (Floragard TKS 2). Seeds of *S. vulgaris* were sown and grown in a greenhouse without additional light at the University of Fribourg (Switzerland). Seeds of *C. bursa-pastoris* were sown and germination was induced by stratification in an incubator with a regime without light of 11 hours at 4°C and 1 hour at 15°C for one week. Relative humidity fluctuated between 70 and 80%. Trays with stratified seeds were moved to the greenhouse and grown under the same conditions as *S. vulgaris*.

The experiment was carried out on a lawn at the University of Fribourg (Switzerland). Two experimental plots of 8.1m by 8.1m were marked. In each plot, 13 by 13 patches of 0.3m by 0.3m were marked. The distance between the patches was 0.3m. Grass was removed from the patches and these were weeded during the whole experiment. The grass around the patches was frequently mown to eliminate the competitive effects of grass.

Fertilisation, transplant and inoculation

Half of the patches was fertilised with 7g N-P-K-Mg (14-7-14-2) fertiliser (Hauert Tardit Langzeitdünger) per patch (= 78g m^{-2}; label instructions) one day before plants were transplanted to the field. Both *S. vulgaris* and *C. bursa-pastoris* were transplanted to the experimental field on 25 May 1999, which was 22 days after sowing. Plants had one or two true leaves at this date. One plant of *C. bursa-pastoris* was planted at the centre of each patch. Eight, four or nil plants of *S. vulgaris* were planted in a regular pattern around *C. bursa-pastoris*. Plants in a patch were inoculated once, either at 3, 9 or 16 days after the

plants were transplanted into the field, or not at all. Five mg aeciospores was suspended in 10 ml water and equally sprayed over the surface of a patch by use of a DeVilbiss sprayer. Water without spores was sprayed onto control patches and patches that were not inoculated that day. All patches in the plots were covered with plastic for one night after inoculation. Each combination of fertilisation, density of groundsel plants and date of inoculation was applied 14 fold and randomly assigned to the patches of a plot.

Data collection

Senecio vulgaris was examined for the presence of open aecia each day. Latent period was determined as the number of days between inoculation of the patch and the date the first open aecium was observed, to confirm that infection observed was caused by the artificial inoculation. Plant height at senescence and survival of one randomly chosen *S. vulgaris* per patch were determined once a week. The pod production per plant of *C. bursa-pastoris* was determined for each patch at the end of the experiment i.e. 49 days after transplanting plants to the field.

Data analysis

Life expectancy of *S. vulgaris* was defined as time in days between transplant and dieback of *S. vulgaris*. Due to lack of homogeneity of variances of the data set the effects of inoculation date, plant density and fertilisation on life expectancy of *S. vulgaris* were analysed using the test of Kruskal-Wallis (Sokal & Rohlf, 1995).

The growth curves of *S. vulgaris* were determined by fitting log-logistic curves to data of plant height using non-linear regression (Frantzen, 1994):

$$y = 1/(1+\exp(-b \times \ln(t/\tau))) \qquad (1)$$

where y = the fraction of maximum plant height, b= a shape parameter, t = the time in days after sowing, and τ the mid height time at which half of the maximum plant height had been reached. Curves were fitted to data of one plant per patch. Effects of fertilisation and time of inoculation with *P. lagenophorae* on shape parameter b and mid height time τ were analysed using the test of Kruskal-Wallis (Sokal & Rohlf, 1995). Effects on growth rate and/or the period a plant continues to grow can be obtained from these two parameters.

Patches without *S. vulgaris* could not be included in the data analysis because the factor 'inoculation' was not valid for patches with only *C. bursa-pastoris*. These patches were added to confirm the reducing effect of *S. vulgaris* towards *C. bursa-pastoris* by competition. Effects of fertilisation, density of *S. vulgaris* and inoculation on number of pods per *C. bursa-pastoris* plant were tested on significance by means of an analysis of variance (ANOVA, Sokal & Rohlf, 1995). A full factorial design was used with 'fertilisation', 'time of inoculation' and 'density of *S. vulgaris*' as factors with two, four and two categories, respectively.

Data of the two plots were pooled in all analyses as no plot effect could be detected.

RESULTS

General

First symptoms of rust disease were observed on *S. vulgaris* 6 days after inoculation. Latent periods determined on plants inoculated at 3, 9 or 16 days after transplant into the field were 12.9 (SE=0.08), 13.2 (SE=0.06) and 12.7 (SE=0.09) days respectively. Aecia were observed on plants in 25 control patches only 28 days after transplant. All plants showed symptoms of disease 35 days after transplant. Disease severity of non-inoculated plants however, was far below severity of inoculated plants (visual observation, data not quantified).

Performance of *S. vulgaris*

Life expectancy of *S. vulgaris* increased with increased delay of inoculation (Figure 1, P<0.001, Kruskal-Wallis test). *Senecio vulgaris* inoculated 3 days after transplant appeared to survive shortest, while all non-inoculated plants were still living at harvest (49 days after transplant). Life expectancy of *S. vulgaris* appeared to be increased by fertilisation, but the effect was only significant when plants were inoculated 9 and 16 days after transplant (P<0.001, Kruskal-Wallis). Life expectancy was also significantly reduced when plants were grown at increased density (Figure 1, P>0.001, Kruskal-Wallis test).

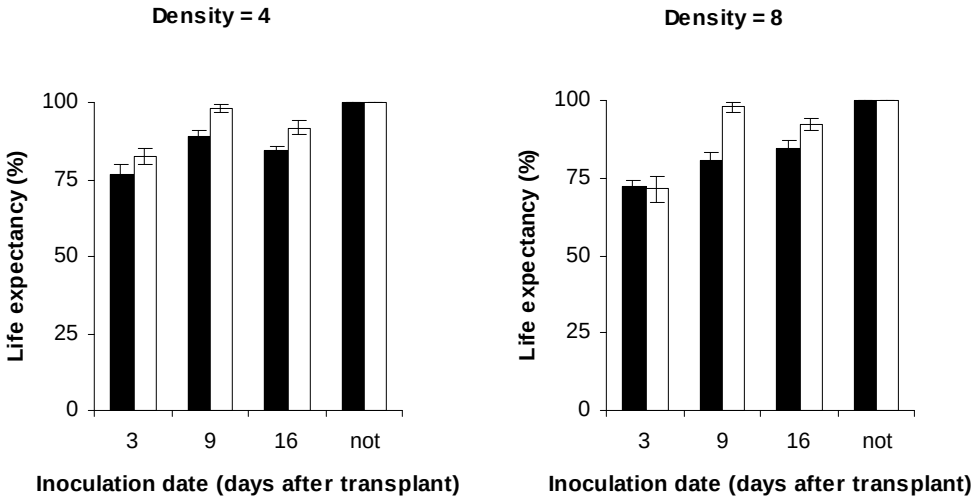

Figure 1: Effect of fertilisation and inoculation with *P. lagenophorae* on percentage life expectancy of S. vulgaris (100% are 49d after transplant, .e. when the experiment was stopped) at densities of 4 and 8 plants per patch. White bars: no fertilisation, black bars: fertilisation

Both time of inoculation of *S. vulgaris* with *P. lagenophorae* and fertilisation had a significant (P<0.001, Kruskal-Wallis) effect on maximum plant height. An interaction between both factors was expected, but could not be tested because homogeneity of variance of the data set was lacking (P<0.01, Levene's test of equality of error variance). Maximum plant height tended to increase with increasing period between transplant and inoculation (Table 1), with non-inoculated plants being clearly tallest and probably still growing at harvest.

Table 1: Maximum plant height of *S. vulgaris* and estimates and standard error (in parentheses) of parameters of a log-logistic curve fitted to the data of plant height of *S. vulgaris* for different inoculation dates of *S. vulgaris*.

inoculation (days after transplant)	Parameters		Maximum plant height (cm)
	b	τ	
3	7.92 (0.6)	37.23 (0.6)	23.97 (1.3)
9	6.97 (0.1)	41.28 (0.5)	31.84 (1.3)
16	7.71 (0.1)	39.31 (0.6)	27.21 (1.5)
not	8.07 (0.1)	44.73 (0.3)	39.57 (0.9)
	P<0.001*	P<0.001*	P<0.001*

* Test of Kruskal-Wallis

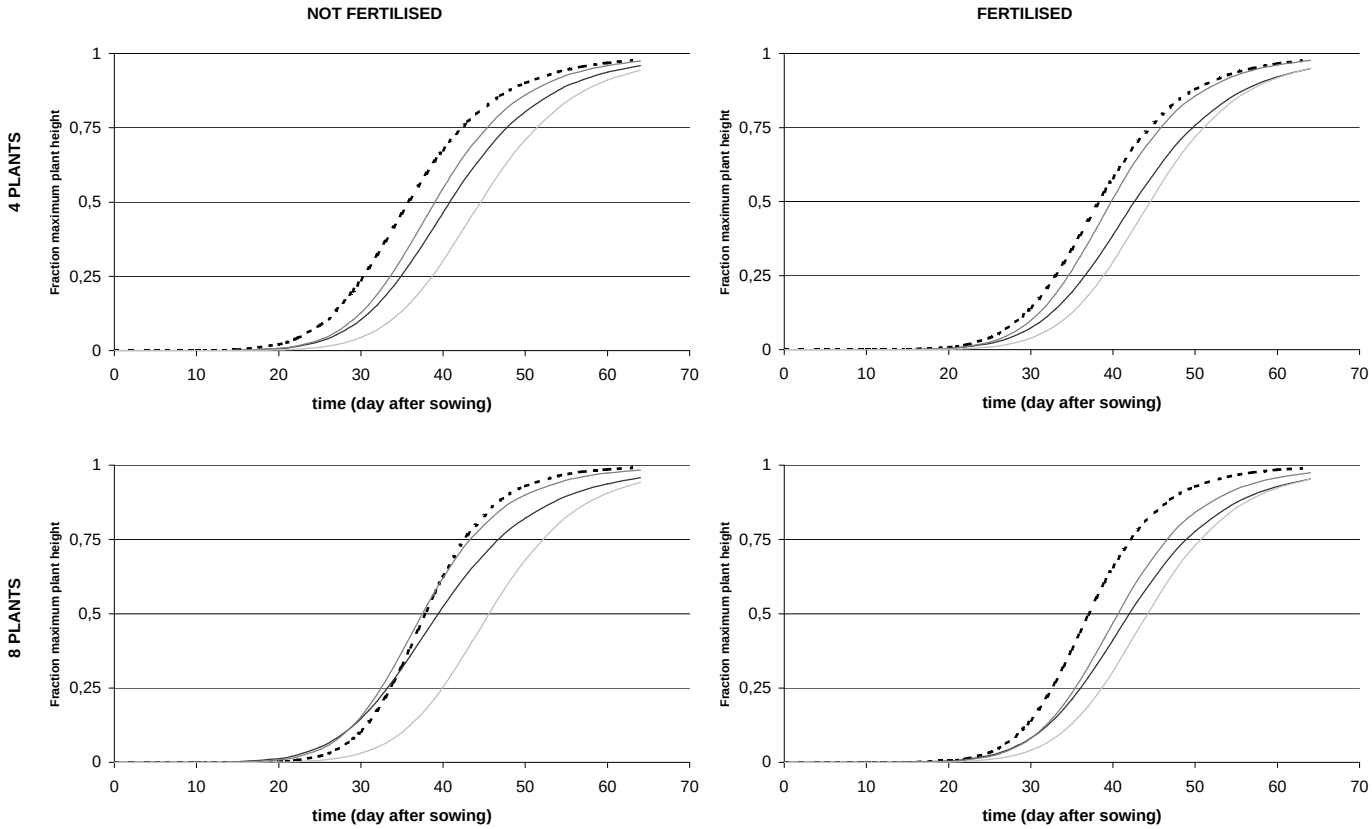

Figure 2: Growth curves of *S. vulgaris*, inoculated with *P. lagenophorae* at 3 (┅), 9 (━) or 16 (━) days after transplant, or not inoculated (━). Curves are best-fitted log-logistic curves. Time 0 indicates the sowing date of *S. vulgaris*. Plants were fertilised or not and grown in a density of 4 or 8 plants.

The growth curves of *S. vulgaris* were affected by inoculation date and were steeper when plants were inoculated as compared to controls (Figure 2). Steepness tended to decrease with increasing period between transplant and inoculation. The mid height time τ of control plants was higher than of plants inoculated 3 or 16 days after transplant. Significant (P<0.001 Kruskal-Wallis) effects of inoculation date on shape parameter *b* and mid height time τ were observed (Table 1). Nutrient addition had a significant (P<0.05) effect on shape parameter *b* only (data not presented).

Pod production of *C. bursa-pastoris*

First flowers and pods of *C. bursa-pastoris* were observed 14 and 21 days after transplant, respectively.

Density of *S. vulgaris*, fertilisation and time of inoculation of *S. vulgaris* significantly affected number of pods produced by *C. bursa-pastoris* (Table 2). Pod production was lower

when *C. bursa-pastoris* was surrounded by *S. vulgaris*, than when grown single (Figure 3). Increased density of surrounding plants further decreased pod production of *C. bursa-pastoris*. Interaction between density of *S. vulgaris* and fertilisation on pod production was demonstrated (Table 2). Fertilisation increased the negative effect of increasing density of *S. vulgaris* on pod production.

A significant (P<0.001; square root transformation of data) effect of inoculation time was observed. Multiple comparisons tests (Scheffé test, Sokal & Rohlf, 1995) demonstrated that pod production of *C. bursa-pastoris* was lower for plants grown between non-inoculated *S. vulgaris* plants than between plants inoculated 3 or 9 days after transplant (Figure 4). Pod production tended to decrease with increasing period between transplant and inoculation of surrounding *S. vulgaris* plants, but this trend was not statistically significant.

Table 2: Effects of *S. vulgaris* density, time of inoculation, fertilisation and their interactions on number of *C. bursa-pastoris* pods (Square root transformation, patches with *C. bursa-pastoris* alone were excluded from analyses).

Factor and interaction	df	F^a
Density	1	34.488***
Time of inoculation	3	12.986***
Fertilisation	1	132.951***
Density * Time of inoculation	3	0.590ns
Density * Fertilisation	1	5.162*
Time of inoculation * Fertilisation	3	0.549ns
Density * Time of inoculation * Fertilisation	3	1.383ns
Error	192	

[a]ns, not significant; *, *P*<0.05; ***, *P*<0.001

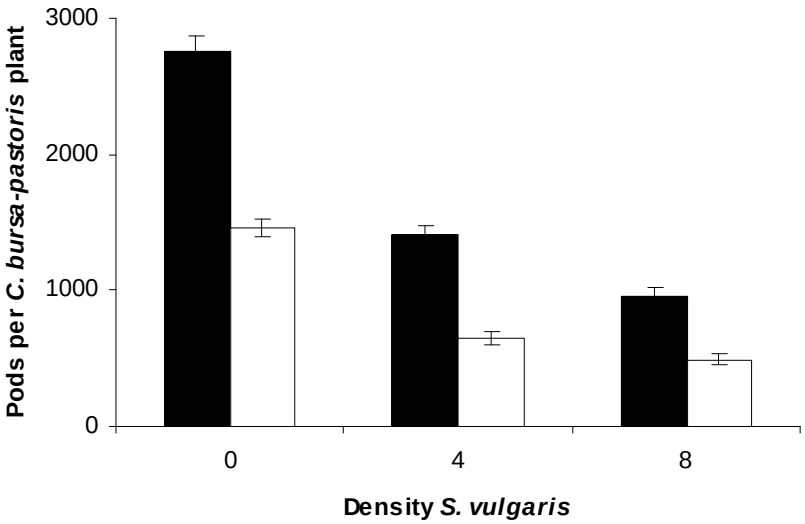

Figure 3: Pod production of *C. bursa-pastoris* grown alone and between *S. vulgaris* (4 or 8 plants). Black bars: fertilised plants, white bars: non-fertilised plants.

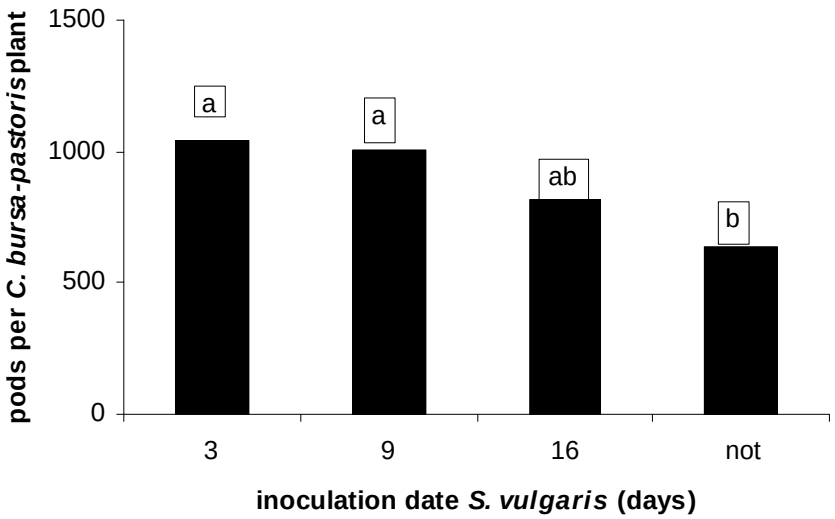

Figure 4: Effect of time of inoculation with *P. lagenophorae* of *S. vulgaris* plants on pod production of *C. bursa-pastoris*. Bars carrying different letters are significantly different ($P \leq 0.05$)

DISCUSSION

Rust infection significantly reduced the growth of *S. vulgaris* and its competitiveness towards *C. bursa-pastoris*. Pod production of the latter species was higher for plants grown between infected *S. vulgaris* than between healthy hosts. Time of infection had a significant effect on growth of *S. vulgaris.* The sooner the plant was infected, the sooner its growth was reduced and the shorter was its life expectancy. This effect of time of infection was not translated into a proportional decrease of competitiveness towards *C. bursa-pastoris* with increased time of infection. But infection of *S. vulgaris* until 9 days after transplant partly compensated the reduction in pod production of *C. bursa-pastoris* caused by competition between the two plant species. Pod production of later inoculated *C. bursa-pastoris* was similar to that of plants grown between healthy *S. vulgaris*. The effect of early host infection on competition was however too small to eliminate competition of *S. vulgaris* towards *C. bursa-pastoris*.

Complete elimination of competitive advantage of *S. vulgaris* over lettuce or *C. bursa-pastoris* by infection of the host plant with *P. lagenophorae* was obtained when the competing species were grown in equal mixtures in pots (Paul & Ayres, 1987a; 1990). A rust epidemic reduced carrot yield loss due to *S. vulgaris* with 30% compared to rust free hosts when both species were also grown in an equal mixture (Grace & Müller-Schärer, 2003). Frantzen (2000) demonstrated an effect of time of host infection on the competitive balance between *S. vulgaris* and celeriac grown in a 2:1 mixture. *Capsella bursa-pastoris* was grown between 4 or 8 competing plants in the present study. The competitive disadvantages to be overcome were thus clearly larger for *C. bursa-pastoris* in the present study than in the other studies.

Crop yield of carrots was increased by introduction of rust inoculum in the field 9-14 days after sowing both crop and weed (Grace & Müller-Schärer, 2003). Early infection of *S. vulgaris* thus has a larger effect on competitiveness when interspecific competitive advantage of *S. vulgaris* is not too large. The critical period of *S. vulgaris* infection to significantly reduce its competitive ability towards *C. bursa-pastoris* ended between 9 and 16 days after transplant (31 and 38 days after sowing respectively) in the present study. The results of Grace & Müller-Schärer (2003) suggested that a larger effect had been obtained when the hosts had been inoculated almost immediately after its emergence. This is thus before inter- and intra-specific competition for space and light between neighbouring plants started.

Growth reduction and senescence of infected *S. vulgaris* was strongly related to time of infection with *P. lagenophorae.* Earlier infections caused larger reductions in plant development and life expectancy and eventually smaller plants, than later infections did. Eventually small plants had steep, early flattening growth curves with shorter mid-height times than eventually larger plants. The reduction in size of infected plants was thus mainly caused by the decreased growth period rather than by a decreased growth rate. This confirmed the earlier senescence of *S. vulgaris* induced by a rust infection as reported by Paul & Ayres (1987c). The earlier *S. vulgaris* was infected, the earlier it died and the more environmental resources (*e.g.* nutrients, space and light) remained available for *C. bursa-pastoris*.

The larger effect of *S. vulgaris* density on pod production at nutrient-rich than at nutrient-poor conditions suggested that *C. bursa-pastoris* used nutrients more efficiently than *S. vulgaris* under nutrient poor conditions. Paul & Ayres (1990) already demonstrated a competitive disadvantage of *S. vulgaris* towards *C. bursa-pastoris* under nutrient-poor conditions. The present study demonstrated that rust infections until 31 days after sowing reduced the competitive ability of *S. vulgaris* towards *C. bursa-pastoris*, but independently of host density or fertilisation. These results suggest that a new *S. vulgaris* population can theoretically be suppressed when a high density of rust spores is available within the first month after emergence of new seedlings in both ruderal and agricultural habitats.

Artificial introduction of *P. lagenophorae* is a way to assure occurrence of a high disease pressure within time. In the present study, *S. vulgaris* was inoculated once to demonstrate the exclusive effect of infection time on growth and competitiveness. Sporulating plants, used as inoculum sources in practice, can remain infective for several days (Chapter 2). The use of inoculum sources instead of spore sprayings can thus further enhance disease pressure considerably and reduce the weed's ability to outgrow the disease. This method was previously used successfully by Frantzen (2000) and Grace & Müller-Schärer (2003) in agricultural systems.

It is generally assumed that plant pathogens are important factors determining the species composition of ruderal and agricultural plant communities. The impact of species specific pathogens on individual plant performance is known for many host-pathogen systems, especially for agricultural crops and their pathogens. Little remains known about their impact on the population dynamics of the host and the resulting species composition of the plant community (Burdon & Leather, 1990). The present study demonstrated that the effect of the species specific pathogen, *P. lagenophorae*, on the performance of its host plant *S. vulgaris* depended on biotic (time of infection, density) and abiotic (nutrient availability) factors. The effect of infection time on host plant performance was however not linearly translated into an effect on competitive ability. The competitive balance of the host plant towards neighbouring *C. bursa-pastoris* was nevertheless equally changed by the earliest two infections, and not by the last one and thus depended on time of infection.

A better understanding of the role of rust pathogens for plant species interactions in natural or semi-natural systems may hopefully lead to further refinement and/or open new possibilities for the deliberate use of these specific pathogens for targeted weed control in crops, as proposed by the system management approach of biological weed control (Müller-Schärer & Frantzen 1996).

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Chapter 6

General discussion

The experiments under controlled conditions of this thesis indicated that both temperature and host susceptibility may affect velocity of epidemic spread of *Puccinia lagenophorae* on a population of *Senecio vulgaris*.

The temperature effect appeared to be mainly caused by its effect on latent period and on the net reproductive number (chapter 2). The latent period of *P. lagenophorae* was moderately sensitive to temperature compared to *Puccinia hordei* on barley (Simkin & Wheeler, 1974), *Puccinia arachidis* on groundnut (Wadia & Butler, 1994) and *Melampsora lini* on *Linum marginale* (Burdon & Elmqvist, 1996). The total aeciospore production per plant, and thus the net reproductive number R_0, increased by almost 50 fold when temperature increased from 10°C to 22°C. This resulted in a three times slower velocity of epidemic spread at 10°C compared to a temperature of 22°C. Low temperatures may thus be one of the causes for the slow build up of epidemics in agricultural and ruderal fields in spring.

Absence of inoculum sources in spring was also suggested to cause a slow build up of epidemics in spring (Leiss & Müller-Schärer, 2001b). Frantzen and Müller-Schärer (1998) proposed artificial introduction of inoculum sources to induce rust epidemics. This thesis indicated that the success of artificially induced epidemics may strongly depend on temperatures. Temperatures previous to rust infection were not demonstrated to affect epidemic development. Contrary to e.g. *Puccinia graminis* on wheat (Brown & Shipton, 1964; Ramage & Sutherland, 1995) and *Phytophthora sojae* in soybean (Gijzen *et al.*, 1996) pre-infection temperature history did thus not appear to affect the susceptibility of *S. vulgaris* to *P. lagenophorae*.

The quantitative resistance of *S. vulgaris* plant lines towards *P. lagenophorae* was revealed in disease severity and aeciospore production, but hardly in latent period (chapter 3). Both the mean time to aeciospore production μ and the standard deviation of the aeciospore production ν were affected by resistance of the plant lines. The plant line effect on velocity of focus expansion appeared to be mainly caused by differences in sporulation period. The effect of quantitative resistance on velocity of epidemic spread may be larger for pathogen systems where resistance is strongly revealed on latent period too, like *e.g. Puccinia recondita* on wheat (Broers, 1989; Wilson & Shaner, 1989; Lehman & Shaner, 1996) *Puccinia hordei* on barley (Parlevliet, 1975) and *Colletotrichum truncatum* on lentil (Chongo & Bernier, 1999).

The resistance of *S. vulgaris* towards *P. lagenophorae* is race-non-specific (Wyss & Müller-Schärer, 1999). The use of a more aggressive rust strain may result in a faster epidemic spread compared to a less aggressive strain. The timing and intensity of inoculum source and the choice of rust strain should thus be adapted to susceptibility of the *S. vulgaris* population towards the rust strain used. Small laboratory experiments, as described in chapter 3 of this thesis could be used for this purpose.

The estimated values of velocity of focus expansion of *P. lagenophorae* in a population of *S. vulgaris* ranged between 3.5 cm day^{-1} at 10°C and 8 cm day^{-1} at 22°C (with a night temperature of 8°C) on plant line pCHu which is relatively resistant to the rust strain used. The velocity of spread of the most susceptible plant line pNLd and the most resistant line pUK differed 1 cm day^{-1}. These estimates lay in the same order of magnitude that was presented as a general estimation by Frantzen *et al* (2001). According to these estimates *P. lagenophorae* is a rather slow expanding fungus (see Zadoks & Van den Bosch, 1994).

The models used to calculated the estimated velocity of epidemic spread from parameters obtained under controlled conditions have been extensively verified and have

been accepted in epidemiology (Levin, 1989; Zadoks & Van den Bosch, 1994). A twice as high velocity was nevertheless observed in the 1998 field experiment of this thesis (chapter 4). The average temperature during the field study was more than 2°C higher than the maximum average temperature in the controlled studies. Temperature may have increased velocity of epidemic spread but this effect alone did not explain the differences between estimated and observed velocity in the studies. The differences in temperature were clearly too small to cause a doubled velocity. Similarly the effect of susceptibility on velocity of epidemic spread was clearly too small to cause this large difference between estimated and observed values.

The estimated net reproductive number R_0 of 383, which is relatively high, and the standard deviation of the contact distribution (or distribution kernel) σ of 28 cm were obtained from an inoculum source consisting of one moderately infected plant (Frantzen & Van den Bosch, 2000). Large changes in R_0 affect velocity of epidemic spread to a small extent only. The velocity is however linearly dependent on σ (Minogue & Fry, 1983a; Van den Bosch, 1988a). The value of σ used to calculate velocity of epidemic spread in the studies under controlled conditions may have been underestimated.

The models used to calculate the estimated velocity of epidemic spread from the parameters obtained under controlled conditions are based on focal expansion like a travelling wave. This means that a closed disease front expands over a host population with a constant velocity (Zadoks & Schein, 1979). Such a focal expansion was observed in the first generation, but disappeared in the second generation of the field study described in chapter 4. Introduction of a *P. lagenophorae* inoculum source in the *S. vulgaris* population created an epidemic that crossed the borders of the field within 2 generations. The power law model fitted the dispersal gradient better than the exponential model. The power law is not exponentially bounded and does not result in a constant wave of focus expansion (Shaw, 1995), but in an exponentially increased velocity of focus expansion over more generations (Frantzen & Van den Bosch, 2000). The second characteristic of a dispersive wave, flattening of the dispersal gradient, was also observed. These results, together with the study of Frantzen & Van den Bosch (2000), suggested that expansion like a dispersive wave is more realistic. The spread like a dispersive wave instead of a travelling wave therefore appears to mainly explain the higher than expected velocity of spread observed in the field.

The spread of a dispersive wave in space, however, is not uniform and time and place of infection are less predictable than for a travelling wave. The more rapid spread of *P. lagenophorae* may thus have disadvantages with respect to providing predictable results needed for a successful use of the system management approach of biological weed control. The first two generations of an epidemic in a young population are, however, the most important using this approach for control of *S. vulgaris* because competitive ability of *S. vulgaris* is mostly reduced during this period (Frantzen, 2000; Grace & Müller-Schärer, 2003; Chapter 5). Predictions of epidemic expansion using travelling or dispersive wave models do not differ that much yet during this period (Frantzen & Van den Bosch, 2000). Until better predictions of dispersive waves are possible, epidemic expansion will therefore be treated as a travelling wave.

The induced epidemic of *P. lagenophorae* on the *S. vulgaris* population did not shift the competitive balance between the host plant and its competitor *Capsella bursa-pastoris* in the 1998 field study (chapter 4). Disease pressure appeared to be too low to decrease the competitive ability of *S. vulgaris* significantly under these conditions. The plant line used, pCHu, is known to be relatively resistant to the introduced rust strain (chapter 3). The same disease pressure on a more susceptible plant line, like pNLd, could have increased any effect on competitive balance. Plant growth and development were not determined in this particular study, and it was not known if they were significantly affected by the rust infection. The single inoculum source introduced caused an infection level of about 120 sori per plant on the nearest plants. This number lay far below the infection level of ± 500 sori per plant that reduced celeriac weight in a field trial by Frantzen (2000). He introduced four inoculum sources instead of one. Besides this low severity the extremely unequal mixture of *S. vulgaris* and *C. bursa-pastoris* (8:1) appeared to mask any effect the rust might have had in the 1998 field experiment.

A different set-up was used in the 1999 field experiment of this thesis (chapter 5). Rust infection was induced by single inoculations with spore suspensions and not by artificial induction of an epidemic. This guaranteed a high disease pressure on the infected host plants. Single inoculations at three different dates were included to determine if later infections were still able to reduce competitive ability of *S. vulgaris.* Host plant growth, development and survival were strongly reduced by the obtained disease pressure. The plants suffered more from early than late infections. The competitiveness towards *C. bursa-pastoris* of hosts infected up to 31 days after sowing was significantly lower than for non-infected hosts. This difference had disappeared for hosts infected later in the study. Although a linear relation between time of infection and shift in competitive balance could not be demonstrated this shifted balance thus clearly depended on time of infection. The shift in competitive balance in the 1999 field experiment was however not enough to change the competitive disadvantage of *C. bursa-pastoris* towards *S. vulgaris* into a competitive advantage. The disadvantage caused by 4 or 8 plants of *S. vulgaris* surrounding *C. bursa-pastoris* appeared to be too high to be overcome by rust infection.

These results indicated that inoculum in the 1998 field experiment (chapter 4) was not introduced too late (28 days after sowing) to cause a shift in competitive balance. The results of Grace & Müller-Schärer (2003) nevertheless suggested that an even larger effect had been obtained when the hosts had been inoculated almost immediately after its emergence. This is thus before inter- and intra-specific competition for space and light between neighbouring plants started.

This thesis clearly demonstrated the effects of temperature and host susceptibility on epidemic development of the rust, but also that a high disease pressure must be obtained in time to reduce competitive ability of *S. vulgaris* towards neighbouring weeds or crops. The reduction levels obtained in this thesis were too low for successful biological weed control in agricultural sites. In more natural sites, artificial introduction of the rust when new host generations start to develop might suppress *S. vulgaris* populations. Population dynamics of *S. vulgaris* are characterised by steep increasing numbers at germination followed by a steep decrease at senescence. Seedling establishment is suggested to be the major factor influencing the development of the next generation (Leiss & Müller-Schärer (2001b). These newly developing generations have to compete with neighbour plants still present at seed germination. The competitive advantage of seedlings in (semi-) natural habitats is thus smaller than in the empty surfaces agricultural sites generally are. Introduction of *P. lagenophorae* might than be enough to change the competitive balance at the expense of *S. vulgaris*. In a similar way introduction of enough inoculum sources at an agricultural site could be more effective when wintering hosts are not completely removed before sowing of the crop. These older plants are more susceptible to the rust than younger ones (Wyss & Müller-Schärer, 1999) and may thus serve as extra 'save havens' for the rust in case of unexpected, temporally deteriorating climatic conditions. Moreover the intra-specific competition from older towards germinating plants may reduce initial competitiveness of the new generation and increase the negative impact of early rust infections. Further research should aim at disease levels needed to suppress host development enough and the effect of *S. vulgaris* densities in plant mixtures on shift in competitive balance with other species.

The data presented in this thesis supported the theory behind the system management approach of biological weed control (SMA) as proposed by Frantzen & Müller-Schärer (1998). It was demonstrated that a rust epidemic could be induced by introduction of an inoculum source in a *S. vulgaris* population. The temporal and spatial development of the first generations of these epidemics were relatively well predicted using data obtained from experiments under controlled conditions. It was also demonstrated that the competitive balance could be shifted away from *S. vulgaris* when a severe rust infection was obtained within a month after seed germination. Several limiting factors were however revealed. Low temperatures and relatively resistant populations were demonstrated to hamper rapid epidemic development. As a result the epidemic was not able to cause a disease pressure severe enough to affect development and competitive ability of the *S. vulgaris* population.

The applicability of the SMA should be further studied including other weed-pathogen systems too. Research should be focused on pathogens that cause enough stress to reduce host development at relatively low infection levels, for example systemic pathogens. This seems to be the most important factor determining the success of the SMA for the moment. Even pathogens that can be used as mycoherbicides should be considered if these pathogens are indigenous and able to form competitiveness reducing epidemics, but for a different reason. The SMA uses indigenous species (Müller-Schärer & Frantzen, 1996) and reduces exposure of the applicator to the pathogen, compared to use of mycoherbicides. Both factors generally form the bottleneck in authorisation of use of micro-organisms in biological control. The SMA might overcome this hurdle for current biological control practises a lot easier than mycoherbicides would.

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