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Studies on the weed pathosystem
Cirsium arvense - Puccinia punctiformis

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Studies on the weed pathosystem *Cirsium arvense - Puccinia punctiformis*

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This thesis contains results of a research project 'Biological control of creeping thistle with the rust fungus *Puccinia punctiformis*'. This project of the DLO-Centre for Agrobiological Research, Wageningen, was supported by the Ministry of Housing and Environmental Protection and the Ministry of Agriculture, Nature Management and Fisheries.

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Stellingen

1. Het groene imago van biologische onkruidbestrijding leidt tot het verontachtzamen van de risico's verbonden aan deze wijze van onkruidbestrijding.

2. Het gebruik van biotrofe schimmels in de biologische onkruidbestrijding is uit oogpunt van risicobeheersing te prefereren boven het gebruik van niet-biotrofe schimmels.

3. Genetische manipulatie van pathogenen wordt als dé oplossing gezien voor de problemen bij het ontwikkelen van biologische onkruidbestrijdingsmiddelen (Sands *et al.* 1990. Weed Technology 4: 471-474). Gelet op het gebrek aan kennis inzake onkruid-pathosystemen (Hasan & Ayres. 1990. New Phytologist 115: 201-222) is het speculeren op genetische manipulatie ten behoeve van biologische onkruidbestrijding een puur theoretische exercitie.

4. Biologische onkruidbestrijding kan een bijdrage leveren aan een duurzame landbouw in Nederland.

5. Manipulatie van de vegetatie is een gemeenschappelijk kenmerk van landbouw en natuurontwikkeling in Nederland.

6. Het verdient aanbeveling om in het onderzoek evenredige aandacht te besteden aan precisie én validiteit van resultaten.

7. Observation in biology has probably produced more insights than all experiments combined (Mayr. 1982. The Growth of Biological Thought: Diversity, Evolution, and Inheritance. The Belknap Press of Harvard University Press, Cambridge).

8. Kansuitspraken met betrekking tot ontsnapping van transgenen hebben alleen maar zin voor eindige tijdsperioden omdat, ook als de kans klein is per tijdseenheid, de kans 1 is dat ontsnapping ooit gebeurt (Van Damme. 1991. Kruisbaarheid van wilde planten en transgene gewassen. In: P Schenkelaars, J Weverling (red.) Verslag Symposium Ecologische Aspecten van Genetisch Gemodificeerde Organismen. Nederlandse Ecologenvereniging - Oecologische Kring, pp. 15-16).

9. Het afschaffen van snelheidscontrôles om de gemiddelde snelheid op de Nederlandse wegen te verlagen kan een praktische toepassing van natuurlijke selectie zijn.

10. Geloven in reïncarnatie leidt tot een groter milieubesef.

Stellingen behorend bij het proefschrift van Jos Frantzen: 'Studies on the weed pathosystem *Cirsium arvense - Puccinia punctiformis*'.

Author's abstract

The biology and epidemiology of the rust fungus *Puccinia punctiformis* (Str.) Röhl was investigated to evaluate the potential of this rust as a biological agent against the clonal plant species *Cirsium arvense* (L.) Scop., which is considered world-wide as a weed. The studies focussed on systemic infection of *C. arvense* shoots by *P. punctiformis*, the most damaging form of infection. The temporal and spatial distribution of teliospores, the inoculum for systemic infection, was quantified to explain the relatively low incidence of systemic infection at the study sites. These studies were extended by experiments under controlled conditions to determine the influence of temperature and resistance on root bud infection, a necessary step to systemic infection. Escape and resistance were factors that reduce the impact of *P. punctiformis* on *C. arvense* populations. Subsequent studies on transport of teliospores in the soil and selection of aggressive *P. punctiformis* strains are proposed as contributions to the development of *P. punctiformis* as a bioherbicide against *C. arvense*.

Additional index words: pathogen, rust fungus, teliospores, disease escape, quantitative resistance, clonal growth, matrix population model, bioherbicide

Voorwoord

Dit proefschrift is een gedeeltelijke weergave van onderzoek dat verricht is binnen het project 'Biologische bestrijding van akkerdistel met de roestschimmel *Puccinia punctiformis*'. In dit proefschrift worden de wetenschappelijke aspecten van het project uitgediept. Een meer volledig, maar globaler, verslag van het project is als CABO-DLO uitgave verschenen (Frantzen & Scheepens, 1993).

Mijn promotor Jan Zadoks is gedurende de jaren van onderzoek en schrijven van het proefschrift een trouwe begeleider geweest van wie ik veel heb geleerd. Mijn co-promotor Bert Lotz was in de eindfase dagelijks aanwezig om mij te steunen. Aan promotor en co-promotor maar ook vele anderen ben ik dank verschuldigd. Deze dank zal ik op een meer persoonlijke wijze tot uiting brengen dan via dit voorwoord mogelijk is.

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CHAPTER 1

General introduction

Biological weed control

Using a collective concept, Van der Zweep (1979) defined weeds as: 'the entire vegetation interfering at a specific locality with the objective of the manager'. Using a species concept, Van der Zweep defined a weed as: 'a plant species of which individuals often occur at localities where they interfere with the objectives of the manager'. Holzner (1978), emphasizing the ecological characteristics, defined weeds as plants adapted to man-made habitats and interfering there with human activities, and a weed species as a plant species that meets the definition in at least a part of its area. In the present thesis the species concept is followed: a weed is a plant species, which individuals often occur at, and are adapted to, man-made habitats, interfering with the objectives of the manager. The term weeds is used as the plural of weed.

Biological weed control is an approach utilizing living organisms to control the population of a weed (Watson, 1991). According to Odum (1971), a population is a group of organisms of the same species, occupying a particular space. A population may be characterized by density, the population size in relation to some unit of space. Density is most often expressed as the number of individuals or biomass per unit space. The density of a population has a maximum, the carrying capacity, at which level all the available resources are utilized by the individuals of the population. The density of a population may fluctuate between the carrying capacity and the zero level. Mostly, the amplitude of fluctuations will be smaller due to natural control (Fig. 1.1, next page). Natural control is defined as the maintenance of population density within certain limits by the action of the whole environment, including an element that is density-induced (regulation), in relation to the conditions of the environment and the properties of the species (Huffaker *et al.*, 1971). Weather is an abiotic, stochastic factor controlling fluctuations, and an occasionally high abundance of a natural enemy may be an example of a biotic, stochastic factor. In contrast to control by stochastic factors, regulation includes the actions of repressive environmental factors, which intensify as population density increases beyond a certain limit, and relax as density is below this level (Huffaker *et al.*, 1971). Decreasing availability of resources with increasing density may be an example of density regulation by abiotic factors. Natural enemies may function as biotic factors, regulating population density.

The classical or inoculative approach to biological weed control involves the introduction of natural enemies from the native area of a weed into areas that were colonized by the weed, but where the natural enemies are absent (Watson, 1991). Introduction of the natural enemies should result in a regulation of the weed population at acceptably low levels. Striking examples of this classical approach are the introduction of the moth *Cactoblastis cactorum* (Berg.) in Australia against

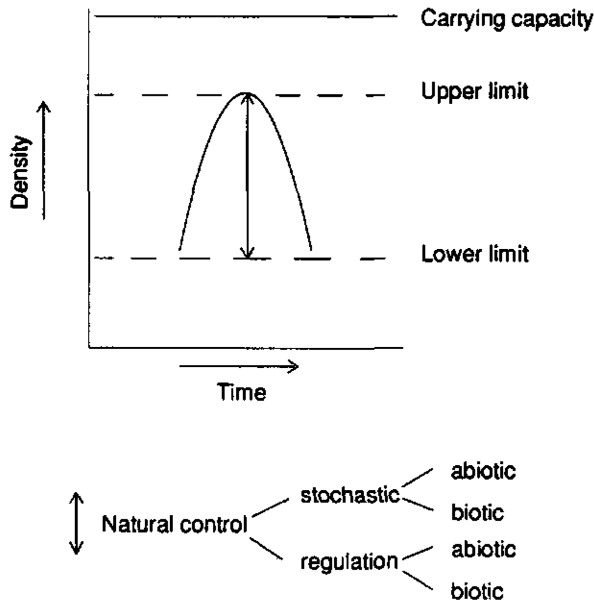

Figure 1.1 Control of plant population density by natural factors in time, adapted from Odum (1971).

Opuntia spp. (reviewed by DeBach & Rosen, 1991) and the introduction of the rust fungus *Puccinia chondrillina* Bubak & Syd. into Australia against *Chondrilla juncea* L. (Cullen *et al.*, 1973; Burdon *et al.*, 1981).

A second approach to biological weed control has been developed for areas where weeds and fungi live in association, but where populations of fungi can not build up sufficiently to have destructive effects on the weeds, the inundative approach (Templeton *et al.*, 1979; Hasan & Ayres, 1990). Fungi are cultured and periodically applied, like herbicides, to control the populations of the target weed. Charudattan (1988) made a subdivision in the inundative method *sensu stricto* or mycoherbicide approach, and the augmentative approach. The former approach is based on the mass production of fungi in fermentors and the subsequent application of relatively large amounts of inoculum onto the target weed. The augmentative method is restricted to fungi that are not mass-produced in fermentors, but are applied in relatively low amounts onto the target weed. After application, incidence of the fungus has to increase by reproduction. The term augmentative approach is, however, also used for a subdivision of the classical approach into the classical approach *sensu stricto* (inoculative), the augmentative approach when the classical biological agent requires a periodic re-establishment, and the inundative approach when introduction is frequently

required and in relatively large amounts (Hasan & Ayres, 1990). To avoid debate about which amount of inoculum has to be considered as augmentative or inundative, how many times a biological agent should be imported to be inoculative, augmentative or inundative, and which taxon of biological control organism is used, I adapted the classification and definitions of biological weed control, given by Templeton (1982) and Watson (1991), for the present thesis:

(1) The classical approach is based on the use of the innate ability of an introduced biocontrol organism to become established in its new habitat and to regulate weed populations at acceptable levels with respect to the objectives of the manager;
(2) The bioherbicide approach is based on the artificial increase of the ability of a biocontrol organism to control weed populations at acceptable levels with respect to the objectives of the manager.

Pathosystems

A pathosystem is defined as any sub-system of the ecosystem which involves parasitism (Robinson, 1976). Parasites may be pathogenic, i.e. cause diseases of plants. Viruses, mycoplasms, bacteria and fungi may be parasites that cause plant diseases. Because of the ability to cause disease, pathogens are used for biological weed control. Biological weed control following the bioherbicide approach is, until now, concentrated on pathogenic fungi.
A pathogenic fungus may be described in terms of dispersal units and infection units (Zadoks & Schein, 1979). A dispersal unit is any device for spread and survival of the fungus (e.g. a spore). When a dispersal unit comes into contact with a suitable plant part, the dispersal unit may change into an infection unit, the mycelial structure (fungal tissue) that originates from a dispersal unit. The infection unit may cause a visible lesion on the infected plant, recognized by a discolouration of the host around the point of entry. The lesion may be localized (local lesion infection), or the fungus may grow away from the point of entry and colonize a large part of the plant, or the whole plant (systemic infection). After some time, an infection unit may produce many dispersal units. The cycle of dispersal units causing infection and infection units producing dispersal units may be repeated manifold in space and time. An epidemic builds up by means of a polycyclic process.
The impact of pathogenic fungi on host populations may be limited by various factors (Table 1.1, next page). Depending on the spatial and temporal distribution of host and pathogen, susceptible tissue of the host may escape from contact with infectious dispersal units of the pathogenic fungus. The term escape has been used in a rather broad sense for every lack of contact between susceptible host tissue and infectious dispersal units of the pathogenic fungus if both are present in the same environment (Agrios, 1980; Dinoor & Eshed, 1984). According to Burdon (1987), the term escape

Table 1.1 Factors that may reduce the impact of a pathogen on the host population. For explanation see text.

Escape
Avoidance
Resistance
Tolerance
Compensation

should be restricted to the lack of contact due to environmental factors. For example, the wind direction may determine in which part of the host population fungal spores are deposited. Avoidance encompasses the mechanisms under genetic control of the host that reduce the contact between susceptible host tissue and infectious dispersal units of the pathogenic fungus (Burdon, 1987; Parlevliet, 1989). For example, closed-flowering barleys prevent contact between stamens and spores of the smut fungus *Ustilago nuda*, whereas open-flowering barleys do not (Agrios, 1980). Infectivity may be defined as the mechanisms under the genetic control of the pathogenic fungus that promote the contact between infectious dispersal units of the pathogenic fungus and susceptible host tissue. Infectivity has not yet been demonstrated.

Resistance operates after establishment of the parasitic contact between pathogenic fungus and host and refers to the genetically controlled mechanisms that interfere with and so reduce the growth and/or development of the fungus (Parlevliet, 1989). The counterpart of host resistance is pathogenicity of the fungus.

Given a certain amount of fungal development, the host plant may suffer more or less damage from infection due to the phenomenon of tolerance. Tolerance is defined by Schafer (1971) as 'that capacity of a cultivar resulting in less yield or quality loss relative to disease severity or pathogen development when compared with other cultivars'. Tolerance is a heritable characteristic. According to Parlevliet (1989), tolerance is difficult to evaluate and may not occur frequently. Burdon (1987) hypothesized that tolerance might be more important in natural plant populations than in agricultural plant populations. So far, tolerance has not yet been demonstrated for wild plants.

Within-plant compensation may be the base for tolerance (Mussell, 1980). The phenomenon of within-plant compensation is well illustrated by the increase of photosynthesis of uninfected parts of a plant that may occur after infection of other parts of the plant (references in Walters, 1985). Beside this within-plant compensation, compensation may exist between conspecific plants (Zadoks & Schein, 1979). Resources not utilized by infected plants, because of a retarded growth, may be available for enhanced growth of uninfected neighbour plants, and death of infected plants may be compensated by development and growth of new plants. At the population level, infection of plants does not necessarily results in a reduction of host density. Here, compensation is defined as any increase of activity of non-infected plant

parts (or plants), triggered by infection of other plant parts (or conspecific plants), that makes up, at least partially, for losses due to infection, at the plant or at the population level.

Objective

Biological weed control following the classical approach has been well developed (DeBach & Rosen, 1991). In contrast, biological weed control following the bioherbicide approach is still in its infancy. World-wide, six bioherbicides are now available (Charudattan, 1991). In Europe, the first bioherbicide has been developed and is waiting for registration to be marketed. This bioherbicide has been developed for control of *Prunus serotina* Erhr. using the fungus *Chondrostereum purpureum* Pers. ex Fr. (De Jong *et al.*, 1990). To formulate principles of biological weed control following the bioherbicide approach, two contrasting pathosystems are currently investigated and compared at the DLO-Centre for Agrobiological Research, *Ascochyta caulina* (P. Karst.) v.d. Aa & v. Kest. on *Chenopodium album* L. and *Puccinia punctiformis* (Str.) Röhl on *Cirsium arvense* (L.) Scop. (Scheepens & Frantzen, 1990). The aim of the present thesis was to determine the effects of the factors shown in Table 1.1 on the impact that *P. punctiformis* exerts on *C. arvense* populations.

Study organisms

The perennial plant species *Cirsium arvense* (L.) Scop. is a common weed world-wide (Donald, 1990). Trivial names of this species are creeping thistle, Californian thistle, Canadian thistle, Ackerkratzdistel (German) and Akkerdistel (Dutch). The species is predominantly dioecious (Lloyd & Myall, 1976) and it may colonize new sites by means of seed (Bakker, 1960). Plants of *C. arvense* produce creeping roots on which buds develop to new aerial shoots (Leackey, 1981).

The biotrophic fungus *Puccinia punctiformis* (Str.) Röhl., a rust fungus (Order *Uredinales*), infects *C. arvense* shoots systemically (systemic infection) or locally (local lesion infection). Biotrophic fungi are fungi that need the living host tissue to grow and reproduce. Since the first description (Persoon, 1799), the rust is described as specific for *C. arvense* by most authors. De Bary (1863) mentioned also *Taraxacum officinale* (L.) Weber as host, but inoculation experiments of Buller (1950) failed to confirm this report. It is difficult to assess whether the deviant report of De Bary (1863) is caused by a mistake of classifying the rust species, occurring on *T. officinale* and *C. arvense*, or that specific races of *P. punctiformis* were involved in the study by De Bary (Buller, 1950).

Outline

An epidemiological study of *P. punctiformis* is presented in Chapter 2, followed by a more extended description of *P. punctiformis* populations at four grassland sites (Chapter 3). The impact of *P. punctiformis* on the population dynamics of *C. arvense* is studied by a matrix population model (Chapter 4). Results of germination experiments under controlled conditions (Chapter 5) were used to conduct experiments testing *C. arvense* clones on resistance (Chapter 6). Chapter 7 discusses transport of *P. punctiformis* teliospores in the soil. The results are integrated and discussed in Chapter 8 with respect to biological weed control in general, and biological control of *C. arvense* specifically.

CHAPTER 2

An epidemiological study of *Puccinia punctiformis* as a stepping-stone to biological control of *Cirsium arvense*

SUMMARY

The infection cycle of the autoecious rust fungus *Puccinia punctiformis* on the clonal plant *Cirsium arvense* was described in terms of intensity (systemic infection) and extensity (local lesion infection) using data of four grassland sites in The Netherlands. The incidence of systemic infection was relatively low and systemically infected shoots were concentrated at a few locations at a site. Incidence of local lesion infection was relatively high and widespread over the sites. Spatial and temporal distribution of teliospores, the inoculum for systemic infection, was quantified to explain the relatively low and spatially heterogeneous incidence of systemic infection. Because of the large impact of systemic infection on *C. arvense* shoots, it is suggested that a spatially homogeneous distribution of teliospores may be a prerequisite for the biological control of *C. arvense* by *P. punctiformis*.

INTRODUCTION

The use of fungi offers prospects for the biological control of weeds, but knowledge about fungi on weeds, and on wild plants in general, is relatively scarce (Hasan & Ayres, 1990). The autoecious rust fungus *Puccinia punctiformis* (Str.) Röhl. seems to be an exception to this general statement.
Since the first description of *P. punctiformis* on its host, the clonal plant *Cirsium arvense* (L.) Scop. (Persoon, 1799), several studies were addressed to this rust. One topic was the infection process leading to systemically infected shoots (Olive, 1913; Buller, 1950; Menzies, 1953; Van den Ende *et al.*, 1987; French & Lightfield, 1990). Gäumann (1959) stated that *C. arvense* buds were infected by the rust resulting in systemically infected shoots. Gäumann's description is confirmed by more recent

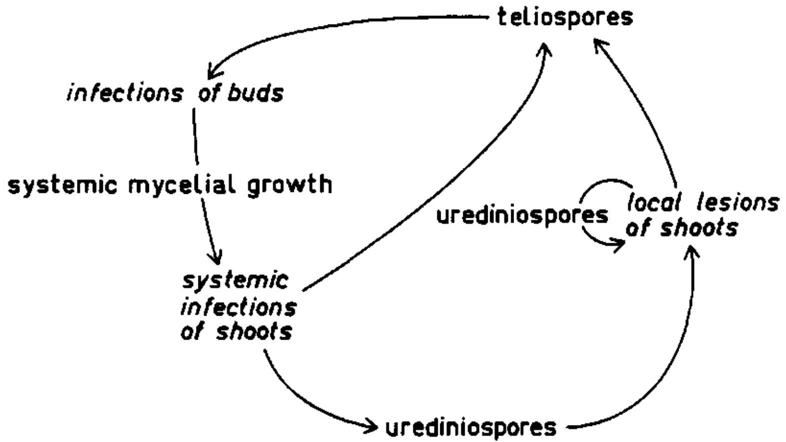

Figure 2.1 Infection cycle of *Puccinia punctiformis* on *Cirsium arvense* after Gäumann (1959). The stages of basidia and pycnia are omitted.

studies (Van den Ende *et al.*, 1987; French & Lightfield, 1990). The infection cycle proposed by Gäumann is, therefore, adopted in the present study (Figure 2.1).
Systemic infection has a strong impact on the host physiology (Kourssanow, 1928; Bailiss & Wilson, 1967), which may result in an early death of systemically infected shoots compared to healthy shoots or shoots with local lesions (Watson & Keogh, 1980). Because of this impact of systemic infection on the host, the rust was tested for its value in biological control of *C. arvense* (Cockayne, 1915; Ferdinandsen, 1923). The former author provided no quantitative data and his work is difficult to judge. Ferdinandsen determined the fraction of systemically infected *C. arvense* shoots at two agricultural sites in the periods 1915 to 1920 and 1916 to 1919, respectively. The fraction systemically infected shoots was determined in a plot of 64 m^2 at each site. At the one site, the fraction increased gradually from 0.04 in the first year to 0.55 in the fourth year, and declined thereafter to 0.18 in the sixth year. The total number of *C. arvense* shoots increased from 860 in the first year to 1395 in the third year and decreased thereafter to 174 in the sixth year. At the other site, the fraction systemically infected shoots changed gradually from 0.0 in the first year to 0.36 in the fourth year. The total number of *C. arvense* shoots was 292 in the first year and 560 in the fourth year. These results suggest that (1) biological control of *C. arvense* using *P. punctiformis* is achievable, and (2) the success depends on site.
Generalization of the results of Ferdinandsen (1923) to develop a method of biological control requires repeating his observations at a larger scale. Such a long term study,

however, is not only time consuming, but also less informative than getting insight in the epidemics of *P. punctiformis*. Because no epidemiological study was conducted until now, the present study may be seen as a first step in the understanding of the epidemics of *P. punctiformis*. The study started with a comparison of the incidence of systemic infection and the incidence of local lesion infection at four sites in The Netherlands. Incidence is here defined as the fraction of *C. arvense* shoots infected by *P. punctiformis*, either systemically or locally on the shoot. Subsequently, spatial processes underlying changes of incidence were studied.

MATERIALS AND METHODS

Sites

In 1989, four grassland sites were selected in The Netherlands (see also Chapter 3). The site Almere had a grass sward dominated by *Festuca rubra* L. and a clay-loam soil. Until 1989, the site was mown in June and in the autumn. The site Dinteloord was a former salt marsh reclaimed in 1986 and had a relatively open vegetation. The site was not subjected to any management until 1989. The site Middelharnis had a wet and peaty soil, and a relatively dense grass sward consisting of several species. Until 1989, the site was mown in June and thereafter grazed by cattle. The site Yerseke had a sandy-loam soil and a less dense grass sward, reflected by the presence of *Elymus repens* (L.) Gould. The site was mown several times a year, grazed by cattle, and *C. arvense* was chemically controlled until 1989. From 1989 on, sites were not managed, except mowing in late June or early July to prevent dispersal of *C. arvense* seed to farmers' land.

Incidence and Gini coefficient

At each site, 24 plots of 4 m by 4 m each were laid out in a pattern of 3 by 8 with distances of 2 m between plots. Shoots in the plots were counted monthly from April to October in 1989, and in June and October of 1990. Shoots were classified as healthy, systemically infected, or bearing local lesions. Differences between sites in incidence of systemic infection and incidence of local lesion infection were tested for significance by the non-parametric test of Kruskal-Wallis (Sokal & Rohlf, 1981). Within-site variation with respect to incidence of systemic infection and local lesion infection was quantified using the Gini coefficient. This measure of inequality, used in economics (Cowell, 1977), was introduced in plant population biology by Weiner & Solbrig (1984) to express size hierarchy of plant populations. Individual plants were the units of measurement in their study. Here, plots were used as the units of

measurement. The Gini coefficient was computed using the formula (Dixon *et al.*, 1987):

$$G = (1 / 2\bar{x}n(n-1)) \sum_{i=1}^{n} (2i-n-1)\, x_i$$

where x_i is the value determined for a unit (plot) i and n is the number of units (plots). The values were sorted from the lowest to the largest. The Gini coefficient has a minimum of zero (all units are equal), and a maximum of 1. The calculated G may be biased for small samples (Weiner & Solbrig, 1984; Dixon *et al.*, 1987). The Gini coefficient is more extensively treated in Chapter 3.

Teliospore deposition gradient

In 1991, the deposition gradient of teliospores was determined at the site Huissen (described in Chapter 4). Three infected *C. arvense* shoots were selected and the circumference of each shoot was marked. The radius of a shoot (distance between stem and circumference) ranged between 15 and 30 cm. Up to a distance of 30 cm from the circumference, *C. arvense* shoots around each of the selected shoots were removed. Spore traps were placed under the leaves of the selected shoots (distance 0 cm), at 5 cm and at 15 cm from the circumference (Figure 2.2). In each spore trap, a nitrate cellulose filter was placed on the gauze. Rain water could flow through the filter into the soil, but spores were left on the filter. Spores were trapped during two periods of four weeks each. The first period was in September, the second period in October. In the first period, three shoots with local lesions and three systemically infected shoots were selected. In the second period only three shoots with local lesions were selected. Systemically infected shoots had all died at this time. At the end of a period of spore trapping, the filters were removed and teliospores in an area of 10.7 mm^2 around the centre of the filter were counted. For each shoot, period, type of infection and distance (0, 5 and 15 cm, Figure 2.2), the mean number of teliospores cm^{-2} was computed. The effect of distance on deposition of teliospores was tested for significance separately for each type of infection using a rank correlation test (M.J.W. Jansen, personal communication). The half distance α was determined by means of an exponential model fitted to the data (e.g. Fitt *et al.*, 1987):

$$\ln y = \ln a - b*d$$

and,

$$\alpha = 0.693 / b$$

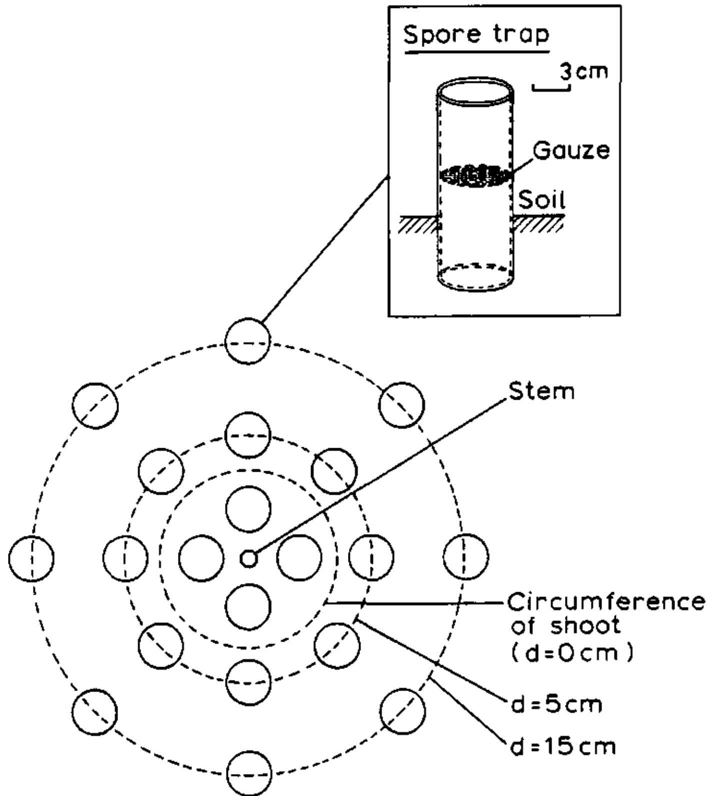

Figure 2.2. Diagram of the design used for trapping teliospores of *Puccinia punctiformis* under the leaves of a *Cirsium arvense* shoot (d = 0), and at 5 and 15 cm from the circumference of the shoot's vertical projection on the soil. In the field, the circumference of the shoot was irregular. An illustration of the spore traps is inserted. Spores were trapped by a filter placed on the gauze.

in which y is number of deposited teliospores, a is a constant indicating the source strength, b is a coefficient indicating the steepness of the gradient, d is the distance from the source, and α is the distance by which the deposition of spores is decreased by one half.

Spatial analysis

In 1992, the spatial distribution of *C. arvense* and *P. punctiformis* was analysed at the site Yerseke. Two plots of 4m by 4m each were selected. The two plots had a different history with respect to *C. arvense* density and incidence of systemic infection (Table 2.1). Distance between the two plots was 38 m. On the 3rd of September, 1992, 13 by 13 cells of 25 cm by 25 cm were marked within each plot. The soil area covered by vertical projection of *C. arvense* shoots, further called coverage, was assessed for each cell using a scale from zero to nine (Table 2.2). To avoid disturbance of the vegetation, the coverage was determined using a scaffold, placed over the plots. On the same day, a spore trap was placed in the middle of each cell, again using the scaffold. Spore traps were removed from plot 1 on the 5th of October, and from plot 2 on the 7th of October. After removal of the spore trap, the soil of a cell was dug out to a depth of 20 cm. Roots of *C. arvense* within each soil volume of 25 cm x 25 cm x 20 cm were collected. Teliospores in the spore traps and the root buds on the collected roots were counted per cell. Teliospores were counted as described above. Data from two cells of plot 1 and four cells of plot 2 were not used in the subsequent analysis because the spore traps in these cells were damaged.
Inequality of the numbers of deposited teliospores and the numbers of root buds within a plot was expressed by the Gini coefficient. The unit of measurement was a cell, 167 cells in plot 1 and 165 cells in plot 2. Spatial autocorrelation, i.e. the probability that the value of a variable measured in a cell is similar to the values measured in neighbouring cells, was determined by two methods, the Moran statistic and geostatistics. The Moran statistic was computed as (Sokal & Oden, 1978):

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

and

$$z_i = x_i - \bar{x} \quad z_j = x_j - \bar{x}$$

in which I is the Moran statistic with a value between -1 and +1, n is the number of cells, w_{ij} a weight that defines two cells i and j as neighbourhood or not, W is the sum of weights, x_i the value of a variable in cell i, and x_j the value of a variable in cell j. Cells were defined as neighbourhoods by distance, in which distance 1 referred to the four cells adjacent to the sides of a cell (Rook's move), distance √2 referred to the four cells adjacent to the corners of a cell (Bishop's move). Whether the I-value differed

Table 2.1 The total number of *Cirsium arvense* shoots (N) and the fraction of shoots systemically infected by *Puccinia punctiformis* (Frsys) in two plots (4m by 4m each) at the site Yerseke in June of four subsequent years.

	1989		1990		1991		1992	
Plot	N	Frsys	N	Frsys	N	Frsys	N	Frsys
1	1	0.00	43	0.07	141	0.11	312	0.05
2	524	0.02	514	0.45	382	0.43	152	0.05

Table 2.2 Scale to determine *Cirsium arvense* coverage[a], adapted from the Braun-Blanquet scale (Westhoff & Van der Maarel, 1973).

Scale value	Coverage
0	*C. arvense* absent
1	< 1 %
2	1 - 5 %, only one leaf present per cell[b]
3	1 - 5 %, a few leaves present per cell
4	1 - 5 %, many leaves present per cell
5	5 - 12.5 %
6	12.5 - 25 %
7	25 - 50 %
8	50 - 75 %
9	75 - 100 %

[a] Coverage is the area of soil covered by shoots of *Cirsium arvense* in vertical projection.

[b] Dimensions of cell 25 cm by 25 cm.

significantly from zero (a spatially random distribution) was tested by a randomization test (e.g. Gilligan, 1986). When an autocorrelation was significant, the analysis was extended to geostatistics, a technique widely used for mapping in geology and pedology and recently also used in plant pathology (Lecoustre *et al.*, 1989). Geostatistics detects spatial dependency by plotting the semivariance of a variable against the distance h between sample units (cells). The semivariance is defined for any distance h by (Lecoustre *et al.*, 1989):

$$G(h) = [1/(2N_h)] * \Sigma[F(x_i+h)-F(x_i)]^2$$

where x_i is one cell of a pair, x_i+h the other cell at h cells from x_i away, F(x) is the value of a variable in a cell, and N_h the number of pairs (x_i, x_i+h). The larger the difference in F at distance h, the higher the value of G(h), the semivariance.

Temporal distribution of teliospores

At the site Yerseke, the temporal distribution of teliospore deposition was determined in 1992. Spore traps were randomly distributed over the site in six groups of nine spore traps each. Distance between two groups was at least 2 m. Distance between the spore traps within a group was 25 cm. Spores were trapped in the period from the 16th of July (day 0) to the 29th of October (day 106). Filters of the spore traps were changed at days 35, 42, 63, 71, and 85. Teliospores were counted as described above. As some spore traps were damaged, means were computed from 6-9 spore traps per group and trapping period, and used for analysis. A log-logistic model (e.g. Campbell & Madden, 1990) was fitted to the data of each of the groups of spore traps:

$$y = c/\{1+\exp(-b*\ln(t/\tau))\}$$

where y is the number of deposited teliospores, c is the upper asymptote of y, b a shape parameter, t is the time in days and τ is the mid time, at which a fraction 0.5 of the maximum number of teliospores (c) is deposited. The rate of teliospore deposition (dy/dt) at the mid time is:

$$v = (b*c) / (4*\tau)$$

RESULTS

Incidence and Gini coefficient

In 1989, the changes in incidence with month of observation were similar at the four sites. From April onward, systemically infected shoots emerged. After mowing in June, systemically infected shoots emerged again. The fraction systemically infected shoots varied only slightly over the months of observation. Local lesion infection was absent in April and May, the fraction of shoots with local lesions was low in June (< 0.1) and increased, after mowing in June, to the highest value in October. Senescence of shoots hampered a quantification of incidence after October.

At all sites and in both years, incidence of systemic infection in June was low compared to the incidence of local lesion infection in October (Table 2.3). The highest fraction of systemically infected shoots was 0.14. According to site and year, incidence of local lesion infection could exceed 0.9 in October. Incidences of, both systemic and local lesion infection differed significantly (Kruskal-Wallis test, $P < 0.001$) between sites in 1989 and in 1990.
In 1989, the Gini coefficient computed for incidence of systemic infection varied only slightly over the months of observation at all sites. The Gini coefficient computed for incidence of local lesion infection was high (up to 1.0) early in the growing season (June), and decreased to the lowest value in October.
At all sites and in both years, the Gini coefficient determined for incidence of systemic infection in June was higher than the Gini coefficient determined for incidence of local lesion infection in October (Table 2.3). The Gini coefficient for incidence of local lesion infection was down to 0.01, indicating that the incidence was nearly the same for all plots. The Gini coefficient for incidence of systemic infection was up to 0.85,

Table 2.3 Incidence and spatial distribution (expressed in Gini coefficient) of *Puccinia punctiformis* on *Cirsium arvense* at four Dutch grassland sites in 1989 and 1990 [a].

		1989		1990	
	Site	Frsys	Frloc	Frsys	Frloc
Incidence					
	Almere	0.04 (193)[b]	0.91 (385)	0.11 (386)	0.95 (391)
	Dinteloord	<0.01 (537)	0.16 (580)	<0.01 (540)	0.04 (313)
	Middelharnis	0.01 (85)	0.52 (47)	0.01 (53)	0.96 (72)
	Yerseke	0.01 (242)	0.97 (308)	0.14 (357)	0.71 (430)
Gini coefficient					
	Almere	0.55	0.02	0.34	0.01
	Dinteloord	0.69	0.47	0.64	0.33
	Middelharnis	0.78	0.13	0.85	0.03
	Yerseke	0.53	0.01	0.40	0.07

[a] Entries are incidence means and Gini coefficients based on 24 plots per site. Fraction systemically infected shoots (Frsys) determined in June and the fraction of shoots with local lesions (Frloc) in October.

[b] Total numbers of shoots in parentheses.

indicating a strong inequality among plots. The differences between the Gini coefficients of systemic infection and local lesion infection were smallest for the site Dinteloord, at which incidence of local lesion infection was relatively low.

Teliospore deposition gradient

Deposition of teliospores around systemically infected shoots showed a trend of decreasing numbers of teliospores deposited with increasing distance from the shoots (Table 2.4). The effect of distance on teliospore deposition was, however, non-significant ($n = 3$, $P > 0.05$). Deposition of teliospores around shoots with local lesions decreased significantly ($n = 6$, $P < 0.001$) with increasing distance. Regressing deposition of teliospores on distance from shoots with local lesions, using the exponential model, resulted in a value of 0.11 for the gradient parameter b and a half distance α of 6.1 cm. The slope of the regression line differed significantly from zero ($P < 0.01$), but only a small amount of variance was explained by the regression equation ($R^2 = 0.35$).

Table 2.4 Deposition of *Puccinia punctiformis* teliospores under leaves of infected *Cirsium arvense* shoots (d = 0), and at 5 and 15 cm from the circumference of the shoot.

			Distance (cm)		
Month	Infection	Shoot	0	5	15
September	Systemic	1	177	56	44
		2	49	24	13
		3	28	16	98
	Local lesion	1	322	42	15
		2	30	15	20
		3	457	83	44
October	Local lesion	1	196	36	35
		2	86	61	15
		3	543	450	107

Entries are the means (cm^{-2}) of four (d = 0) or eight (d = 5 cm and d = 15 cm) spore traps, data of 1991.

Spatial analysis

In plot 1, the coverage by *C. arvense* was in category 6 or higher, except for a few cells (Figure 2.3A, next page). Deposition of teliospores was relatively high and unequally distributed over the plot, the latter expressed in the Gini coefficient (Table 2.5). Cells with high and low numbers of teliospores deposited, respectively, were not randomly distributed as illustrated by Figure 2.3B, expressed in the Moran

Table 2.5 Spatial distribution of *Puccinia punctiformis* teliospores and *Cirsium arvense* root buds in two plots at the site Yerseke[a].

	Statistic	Plot 1	Plot 2
Teliospores			
	Mean number (cm^{-2})	1523	190
	Gini coefficient	0.50	0.57
	Moran's I[b]		
	- Rook's move[c]	0.45^{***}	0.03^{ns}
	- Bishop's move	0.41^{***}	0.02^{ns}
Root buds			
	Mean number ($cell^{-1}$)	6.0	2.7
	Gini coefficient	0.38	0.41
	Moran's I[b]		
	- Rook's move[c]	0.06^{ns}	0.10^{*}
	- Bishop's move	0.01^{ns}	0.02^{ns}

[a] Means, Gini coefficients and Moran's I are based on 167 cells (25 cm by 25 cm each) in plot 1 and 165 cells in plot 2.

[b] Significance tested by a randomization test, ns = non significant , * = P < 0.05, *** = P < 0.001.

[c] Direction of comparison horizontally and vertically (Rook's move) or diagonally (Bishop's move).

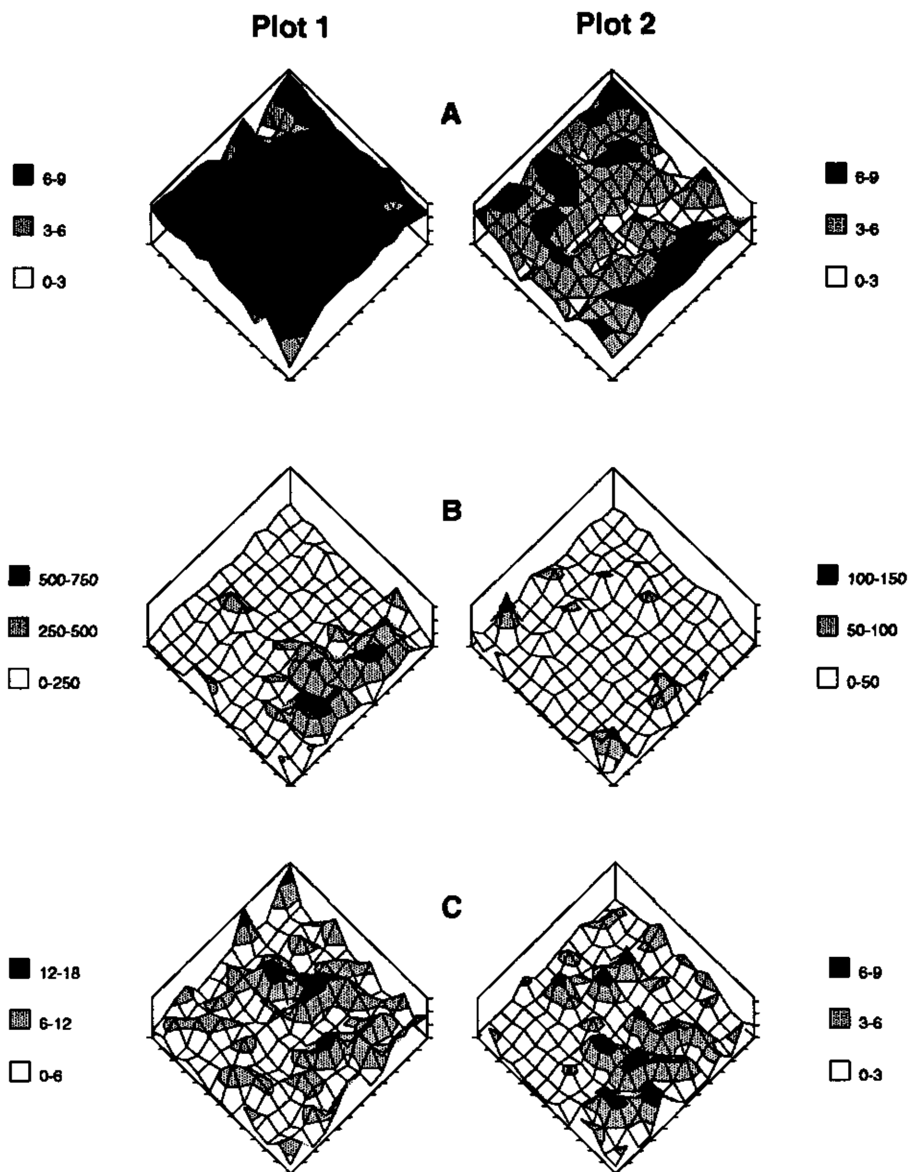

Figure 2.3 Spatial patterns of (A) *Cirsium arvense* coverage, (B) deposition of *Puccinia punctiformis* teliospores and (C) *Cirsium arvense* root buds in two plots at Yerseke in 1992. Data of 13 by 13 cells of 25 cm by 25 cm each, coverage of each cell scored on a scale from zero to nine (Table 2.2), teliospore deposition per cell expressed in cm^{-2}, and the number of root buds collected from the soil of each cell to a depth of 20 cm. Note that the scales for B and C differ between plots.

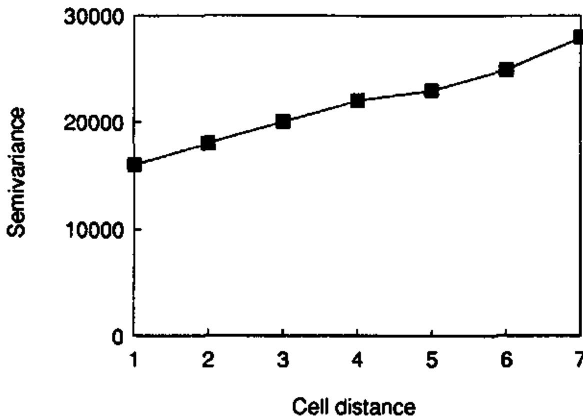

Figure 2.4 Semivariogram of deposition of *Puccinia punctiformis* teliospores in plot 1 at the site Yerseke in autumn 1992. The plot was divided in 13 by 13 cells of 25 cm by 25 cm each. Abscissa is distance expressed in cells, the ordinate is the semivariance (see text).

statistic (Table 2.5) and expressed in the semivariogram (Figure 2.4). The latter shows that the inequality between cells increases with increasing distance between these cells. The number of root buds was relatively high, and varied between cells as expressed in the Gini coefficient (Table 2.5). No evidence for a non-random distribution of cells with a relatively high and low number of root buds, respectively, was obtained.
In plot 2, most cells had a coverage in the class 6 or lower (Figure 2.3A). Deposition of teliospores was relatively low and unequally distributed over the plot, the latter expressed in the Gini coefficient (Table 2.5). The low value of the Moran statistic indicated a random distribution of cells with low and high numbers of teliospores deposited. A point illustrated by Figure 2.3B. The number of root buds was relatively low and varied between cells as expressed in the Gini coefficient (Table 2.5). The Moran statistic indicated a non-random spatial pattern of root buds along the horizontal and vertical axes (Rook's move). Figure 2.3C also suggested a non-random spatial pattern of root buds. Semivariograms (not presented), however, did not provide evidence for an aggregated pattern of root buds.

Temporal distribution of teliospores

The log-logistic model could be fitted to five of the six groups of spore traps (Table 2.6, next page). For these five groups, the estimated values of the maximum number of teliospores deposited (c) varied around a mean of 987 teliospores cm^{-2}. The

Table 2.6 Parameters of a log-logistic model [a] fitted to data of the temporal distribution of *Puccinia punctiformis* teliospores, trapped in six groups of spore traps at the site Yerseke in 1992.

	Parameters		
Group	c (teliospores cm^{-2})	τ (days)[b]	v (teliospores cm^{-2} day^{-1})
1	1149	76	40.1
2	1431	91	9.4
3 [c]	-	-	-
4	804	79	16.3
5	664	73	20.0
6	887	80	24.4

[a] $y = c/\{1+\exp(-b \cdot \ln(t/\tau))\}$ where y is the number of deposited teliospores, c is the upper asymptote, b is a shape parameter, t is the time in days after the 16th of July, and τ is the mid time ($y = 0.5 \cdot c$); the rate of teliospore deposition (dy/dt) at the mid time is: $v = (b \cdot c)/(4 \cdot \tau)$.

[b] The number of days after the 16th of July.

[c] Log-logistic model could not be fitted to the data of this group.

mid deposition time τ varied around a mean of 80 days, i.e. the 3rd of October. The mean rate of spore deposition at the mid deposition time (v) was 22 teliospores cm^{-2} day^{-1}. The data of group 2 suggested that teliospore deposition extended several months after the mid deposition time. The cumulative teliospore deposition determined by the spore traps of group 3 did not level off on the last date of observation, a prerequisite to fit the model adequately. The data of group 3 also suggested an extended period of teliospore deposition.

DISCUSSION

A pathogen population can be described by the spatial attributes of intensity and extensity (Zadoks & Schein, 1979). Intensity is the amount of pathogen per plant or per unit area, and extensity is the area occupied by the pathogen and the distribution of the pathogen within this area. The infection cycle of *P. punctiformis* may be described in terms of intensity and extensity using the results of the present study. In the spring, the rust was concentrated in a relatively few systemically infected shoots, and these diseased shoots were also concentrated at a few localities at a site. The systemically

infected shoots functioned as primary sources of inoculum (urediniospores) for a polycyclic process (Zadoks & Schein, 1979) by which the number of shoots with local lesions increased. At the end of the growing season, over 90% of the shoots had local lesions at some of the sites. The local lesion infection was distributed over the greater part of the *C. arvense* populations.

Systemic infection may be characterized by intensity, local lesion infection by extensity. The loss of extensity of the *P. punctiformis* populations at the transition from the autumnal local lesion infection into systemic infection in the spring may be explained by one or more of the following hypotheses (1) the probability of contact between root buds and teliospores is lower than the probability of contact between urediniospores and shoots, (2) favourable abiotic conditions for the infection process are spatially more heterogeneously distributed for systemic infection than for local lesion infection, (3) more resistance in a *C. arvense* population to root bud infection and subsequent development of systemic infection than resistance to local lesion infection. Data of the present study, directed to the spatial distribution of teliospores, agree with the first explanation.

Dispersal of fungi encompasses three components: spore liberation (from the source), spore displacement and spore deposition (Zadoks & Schein, 1979). In the present study, only the deposition of *P. punctiformis* teliospores was determined. The estimated half distance α of 6.1 cm suggested a rather steep dispersal gradient of *P. punctiformis* teliospores. Because spore dispersal is a complex process (e.g. Fitt *et al*, 1989), experiments under controlled conditions are required to evaluate whether a relatively steep dispersal gradient is to be expected for *P. punctiformis* teliospores. If so, teliospores are predominantly deposited on soil covered by infected *C. arvense* shoots.

The spatial analysis of teliospore deposition at the site Yerseke demonstrated a difference in pattern of teliospore deposition between the two plots investigated. Teliospore deposition in the plot with the highest number of deposited teliospores had an aggregated pattern. The semivariogram of teliospore deposition was similar to a semivariogram for the leaf rust *Puccinia arachidis* Speg., derived from data of a 12 m by 12 m plot (Lannou & Savary, 1991). Lannou and Savary explained the semivariogram by focus formation. A focus is a point of high pathogen concentration surrounded by points of decreasing pathogen concentration with increasing distance (Zadoks & Schein, 1979). Focus formation results from dispersal of a pathogen in time. In a continuous process, inoculum is dispersed from the primary source (e.g. an infected plant) to neighbouring plants, which are, with some delay, the inoculum sources for infection of their neighbour plants. Because of the delay in time, infection is most severe around the primary focus and less at a larger distance. The focus formation determined in the present study may be explained by systemically infected shoots as primary sources of urediniospores causing local lesion infection on neighbouring shoots, and from these shoots the local lesion infection spreads further. The subsequent production and deposition of teliospores reflects, and in a way fixates,

the spatial pattern caused by dispersal of *P. punctiformis* urediniospores in time. The time needed for spread and intensification of local lesion infection may, therefore, have limited the spatial distribution of teliospores. In contrast, the data of the other plot at Yerseke provided no evidence for focus formation. Cells with relatively high and low numbers of deposited teliospores, respectively, were randomly distributed over the plot. The time needed for spread and intensification of local lesion infection did not seem to be the major factor causing the inequality of teliospore deposition in this plot. Spatially heterogeneous biotic or abiotic conditions may have influenced the development of local lesion infection or the subsequent development of teliospores in this plot.

The spatially unequal distribution of teliospore deposition in both plots suggested that the probability of contact between teliospores and root buds varied within the plots, provided that the spatial pattern of teliospores in the soil reflects the pattern of teliospore deposition on the soil.

To study the spatial distribution of teliospores in relation to the spatial distribution of root buds, more knowledge is required about the transport of teliospores in the soil. Transport of teliospores in the soil is, however, an unexplored topic, and rather difficult to quantify (Chapter 7). This lack of knowledge about teliospore transport delays the interpretation of the temporal pattern of teliospore deposition. The temporal distribution of teliospores determined in the present study was one of an onset of deposition in July, and a cessation near the end of October. This pattern suggests that the probability of contact between root buds and teliospores differs in time, unless transport of teliospores in soil influences this pattern. Because French and Lightfield (1990) provided evidence that, under controlled conditions, dormant root buds are susceptible to root bud infection and expanding root buds are not, the temporal distribution of teliospores may have, together with the temporal component of root bud development, meaning for root bud infection.

The present study contributes to the understanding of the epidemics of *P. punctiformis*. Some items of interest were not or not sufficiently considered. The spatial and temporal analysis of teliospore distribution was limited to one of the four sites. The results of similar analyses of the other sites might have been different. A common feature of the four sites was the low and spatially unequal incidence of systemic infection compared to incidence of local lesion infection. The results of the present study suggested the spatially heterogeneous distribution of teliospores as an explanation for the spatially unequal and low incidence of systemic infection. A spatially homogeneous distribution of teliospores may, therefore, be a prerequisite to achieve a sufficiently high level of systemic infection incidence for biological control of *C. arvense*.

CHAPTER 3

Measurement and meaning of hierarchy in pathogen populations

SUMMARY

The spatial structure of *Puccinia punctiformis* populations was determined at four grassland sites in The Netherlands. The Gini coefficient was used to analyse spatial structure, and the term hierarchy was introduced for pathogen populations. Small *P. punctiformis* populations were more hierarchically structured than large populations. At three of the four sites, hierarchy of *P. punctiformis* in the phase of systemic infection was stronger than in the phase of local lesion infection. The biological meaning of hierarchically structured pathogen populations is discussed.

INTRODUCTION

Infection by pathogens reduces the ability of host plants to compete with plants of other species (Paul, 1989; Paul & Ayres, 1987b, 1990). Altering interspecific competition, pathogens might contribute to diversity of plant communities as first understood by Harper (1977). Still, the role of pathogens in plant communities is unclear (Harper, 1990).

Parker (1986) demonstrated that the fungal pathogen *Synchytrium decipiens* was unequally distributed within the populations of its host *Amphicarpaea bracteata*. The greater part of the host plants was uninfected or relatively slightly infected, and a few plants were relatively heavily infected. The mean infection intensity was similar in the two host populations studied, but the distribution of infection intensity differed between the two populations. Intensity of infection was negatively correlated with fitness components of the host. Considering the infections within the host population as a pathogen population, Parker's study revealed that: (1) pathogen populations may have a hierarchical structure, (2) hierarchical structure of pathogen populations is relevant to negative effects on host populations.

To compare pathogen populations in hierarchy of spatial structure, an appropriate measure is required. In the present study, the use of the Gini coefficient was explored

with respect to pathogen populations. The Gini coefficient was introduced in plant population biology by Weiner & Solbrig (1984) to express size hierarchy of plant populations. To make the concept of size hierarchy operational for spatial structure of pathogen populations, the attributes given by Weiner & Solbrig (1984) should be generalized. The more a population structure is hierarchical, the more the following attributes apply: (1) the population contains variation in sizes of units, (2) there are relatively few large units and many small ones, and (3) these large units contribute comparatively much to the overall size of the population. The term units is chosen in stead of individuals as used by Weiner & Solbrig (1984), to choose the most appropriate measure with respect to the purpose of a study, e.g. infections per plant or infected plants per plot.
Parker's study (1986) dealt with a non-systemic pathogen, measured at one time. In contrast, the subject of the present study was a pathogen with a systemic and a non-systemic phase, measured at several times.

METHODS

General

The brachy-form, autoecious rust *Puccinia punctiformis* (Str.) Röhl., which infects the clonal perennial *Cirsium arvense* (L.) Scop., was studied. Systemic infections of shoots, first appearing in April or May (e.g. Gäumann, 1959), function as the primary sources of inoculum for the polycyclic process (Zadoks & Schein, 1979) by which the number of local lesions on shoots is built up (Figure 3.1). Teliospores are produced on systemically infected shoots and on shoots with local lesions.
In 1989, four sites were selected in The Netherlands. Criteria of site selection were occurrence of host and pathogen, and differences between sites with respect to biotic and abiotic conditions (Table 3.1, 3.2). Sites were at least 25 km apart. Until 1989, *C. arvense* plants at these sites were exposed to frequent mowing, trampling by cattle, and, at Yerseke, to chemical control. Only Dinteloord, a former salt marsh, had not been subjected to any form of management since its reclamation in 1986. In 1989 and 1990, sites were only mown in late June or July to prevent dispersal of *C. arvense* seeds to farmers' land. At each site, plots of 4 m by 4 m were laid out in a pattern of 3 by 8 within a study area of 20 m by 50 m. Distances between plots were 2 m.

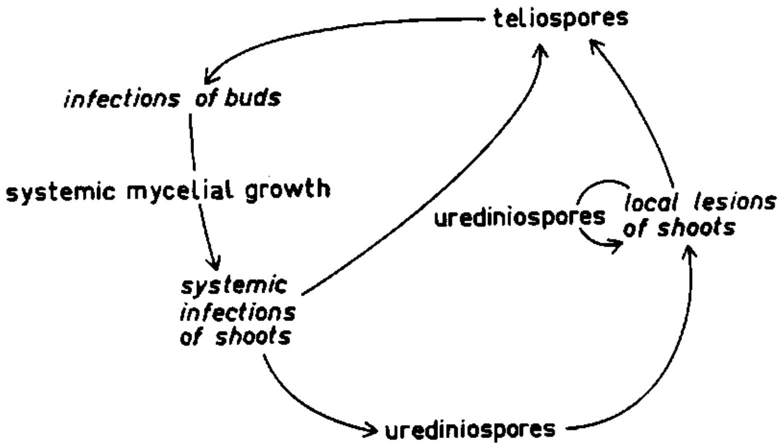

Figure 3.1 Infection cycle of *Puccinia punctiformis* on *Cirsium arvense* after Gäumann (1959). The stages of basidia and pycnia are omitted.

Table 3.1 Characterization of the soil of four sites in The Netherlands, used in the study of the pathosystem *Cirsium arvense - Puccinia punctiformis*.

Site	Texture	pH (KCl)	Organic matter	Phosphate (PAL)	Total nitrogen
Almere	clay-loam	7.3	2.7	0.009	0.16
Dinteloord	sand	7.8	0.7	0.017	0.04
Middelharnis	clay-loam	6.6	12.2	0.012	0.52
Yerseke	sandy-loam	7.3	5.0	0.104	2.26

Data of 1989; contents expressed in g / 100 g ovendry soil.

Table 3.2 Vegetation characteristics of four experimental sites in the Netherlands, used for the study of the pathosystem *Cirsium arvense - Puccinia punctiformis*.

Site	Species	Coverage
Almere	*Festuca rubra* L.	0.73
	Cirsium arvense (L.) Scop.	0.11
Dinteloord	*Ammophila arenaria* (L.) Link	0.15
	Chamerion angustifolium (L.) Holub	0.10
	Cirsium arvense	0.11
Middelhamis	*Agrostis capillaris* L. / *Agrostis stolonifera* L.	0.33
	Lolium perenne L.	0.21
	Hordeum secalinum Schreber	0.18
	Poa trivialis L.	0.17
	Cirsium arvense	0.02
Yerseke	*Elymus repens* (L.) Gould	0.24
	Agrostis capillaris L. /*Agrostis stolonifera L.*	0.21
	Poa trivialis L.	0.19
	Lolium perenne L.	0.18
	Cirsium arvense	0.09

Data of spring, 1990; fraction of soil covered by stems of the most abundant plant species and *Cirsium arvense*; values are means of 24 plots of 4 m by 4 m each.

Pathogen populations

A population of *P. punctiformis* consisted of systemic infections, local lesions and teliospores within the study area. These entities were clustered to larger units to measure the spatial structure of the populations: the number of infected shoots in a plot, the number of local lesions on a shoot, and the amount (mg) of teliospores on an infected shoot.

Infected shoots in the plots were counted in June, September and October of 1989 and 1990, respectively. Shoots were classified as systemically infected or carrying local lesions. In September and October, 1989, 10 shoots with local lesions were collected outside the plots. The lesions on each shoot were counted. In September and October, 1990, shoots with local lesions and systemically infected shoots were collected, each month five samples of 10 shoots with local lesions, and five samples of one systemically infected shoot. Shoots were sampled outside the plots. The amount of

teliospores per sample was determined. The mean value of each of the 16 variates of a pathogen population was computed.
The Gini coefficient was computed for each variate, using the formula (Dixon *et al.*, 1987):

$$G = (1 / 2\bar{x}n(n-1)) \sum_{i=1}^{n} (2i-n-1)\, x_i$$

where x_i are the sizes of the units of a variate, sorted from smallest to largest, and n is the number of units. The Gini coefficient has a minimum of 0 when all units are equal, and, a maximum of 1, the ultimate of hierarchy. At Dinteloord, the collected amount of teliospores was too small to detect variation among the samples of three of the four dates of collection. At Middelharnis, no systemically infected shoots bearing teliospores were found in September, 1990.

Host populations

Spatial structure of *C. arvense* populations was considered as a factor influencing the spatial structure of the pathogen populations. Size and spatial structure of host populations was determined by counting the number of *C. arvense* shoots in the plots. Shoots were counted in June, September and October of 1989 and 1990. The mean and the Gini coefficient was computed for each of the counts.

Statistical analysis

The concept of variability profiles (Sokal & Braumann, 1980) was adopted for statistical analysis. Here, a profile of the pathogen population consists of the mean values, or the values of the Gini coefficient, for the set of variates measured. To achieve an appropriate scaling, the highest mean value of a variate among sites was set to 1, and the values of the other three sites were expressed as fraction of the maximum.
A profile of the host population consists of the mean values, or the values of the Gini coefficient, for the set of counting dates.
Profiles were tested for significant differences between sites, using Friedman's Test for Randomized Blocks (Sokal & Rohlf, 1981).

RESULTS

Pathogen populations

Mean values of the variates of *P. punctiformis* were highest at Almere or Yerseke (Table 3.3). The profile of the mean values of the 16 variates differed significantly between sites (Figure 3.2, Table 3.4A). The *P. punctiformis* populations at Almere and

Table 3.3 Highest mean values of sixteen variates of *Puccinia punctiformis* populations among four sites in The Netherlands.

Number[a]	Variate		Site	Value
1	Ns[b]	June 1989	Almere	8
2	Ns	September 1989	Yerseke	17
3	Ns	October 1989	Almere	14
4	Ns	June 1990	Yerseke	57
5	Ns	September 1990	Yerseke	60
6	Ns	October 1990	Yerseke	100
7	Nl[c]	September 1989	Yerseke	384
8	Nl	October 1989	Almere	351
9	Nl	September 1990	Almere	490
10	Nl	October 1990	Almere	373
11	Ll[d]	September 1989	Almere	301
12	Ll	October 1989	Almere	1303
13	Ts[e]	September 1990	Almere	5.3
14	Ts	October 1990	Almere	1.7
15	Tl[f]	September 1990	Almere	1.1
16	Tl	October 1990	Yerseke	1.0

[a] The numbers are used in Figure 3.4 and 3.5 to indicate the variates on the X-axis
[b] Number of systemically infected shoots per plot of 4 m by 4m; mean of 24 plots
[c] Number of shoots with local lesions per plot of 4 m by 4 m; mean of 24 plots
[d] Number of lesions per shoot with local lesions; mean of 10 shoots
[e] Amount of teliospores per systemically infected shoot in mg; mean of 5 shoots
[f] Amount of teliospores per shoot with local lesions in mg; mean of 5 samples with 10 shoots each.

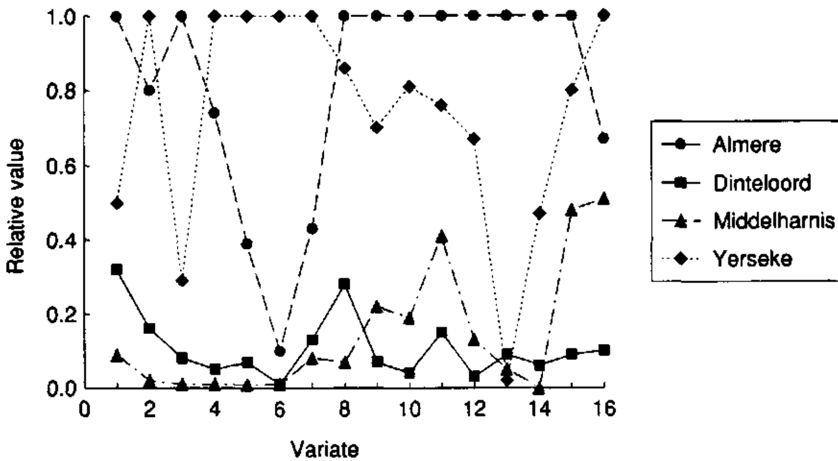

Figure 3.2 Sixteen variates of *Puccinia punctiformis* populations at four sites in the Netherlands. Entries are fractions of the values presented in Table 3.3. Numbers of variates refer to Table 3.3.

Table 3.4 Ranking of four sites in The Netherlands with respect to the profile of mean values (A) and the profile of the values of the Gini coefficient (B) of various variates of *Puccinia punctiformis* populations. In (A) all the 16 variates of Table 3.3 were used, in (B) the variates 1-13. Significance of ranking was estimated with Friedman's Test for Randomized Blocks.

	Site	Rank	
A. Mean values	Almere	3.63	
	Yerseke	3.25	n = 16
	Dinteloord	1.69	$X^2 = 34.7$
	Middelharnis	1.44	$P < 0.001$
B. Gini coefficient	Middelharnis	3.77	
	Dinteloord	2.54	n = 13
	Yerseke	2.15	$X^2 = 20.7$
	Almere	1.54	$P < 0.001$

Yerseke were large compared to those at Dinteloord and Middelharnis. No large differences were determined between the populations at Dinteloord and Middelharnis, except for the amount of teliospores on shoots with local lesions (variate 16 in Figure 3.2). Relatively large differences were determined between the populations at Almere and Yerseke for variates connected to systemic infection (variates 3, 6 and 13 in Figure 3.2).

In Figure 3.3, a typical example demonstrates the relationship between the distribution of the units of a variate and the value of the Gini coefficient. All three distributions were asymmetrical. With respect to the phase of systemic infection in October, 1990, dependency of population size on a few plots was lowest at Almere and highest at Middelharnis. At Almere, the plot with the highest number of systemically infected shoots contributed only about 10 % to the size of the population, but at Middelharnis as much as 50 %, i.e. relatively little hierarchy at Almere and strong hierarchy at Middelharnis.

Most values of the Gini coefficient were about 0.5 or higher, indicating a relatively strong hierarchy (Figure 3.4). Highest values were determined for variates connected to

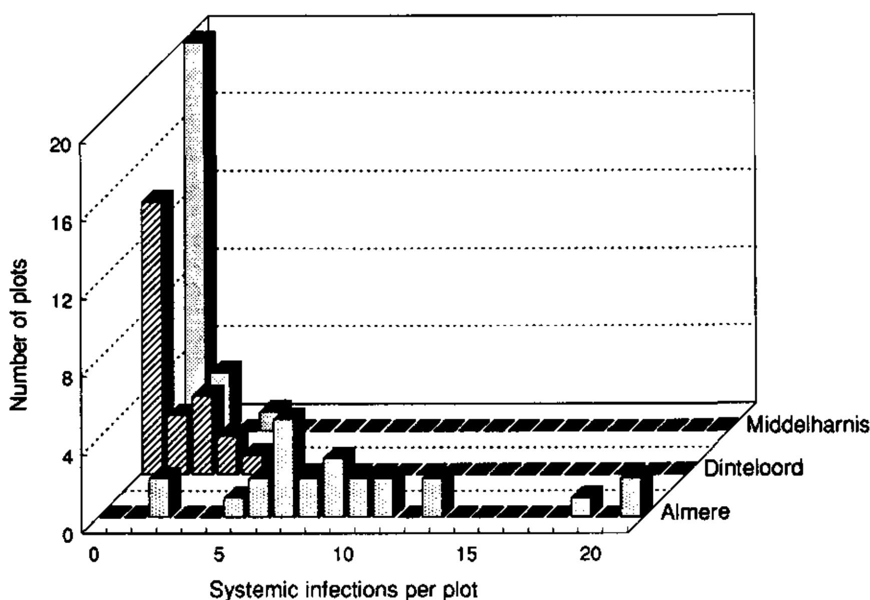

Figure 3.3 Distribution of the number of systemically infected shoots per plot (4 m by 4 m) at three sites in The Netherlands with 24 plots at each site. Values of the Gini coefficient and (means) are for Almere, 0.28 (9.5), Dinteloord, 0.71 (0.9), Middelharnis, 0.91 (0.3).

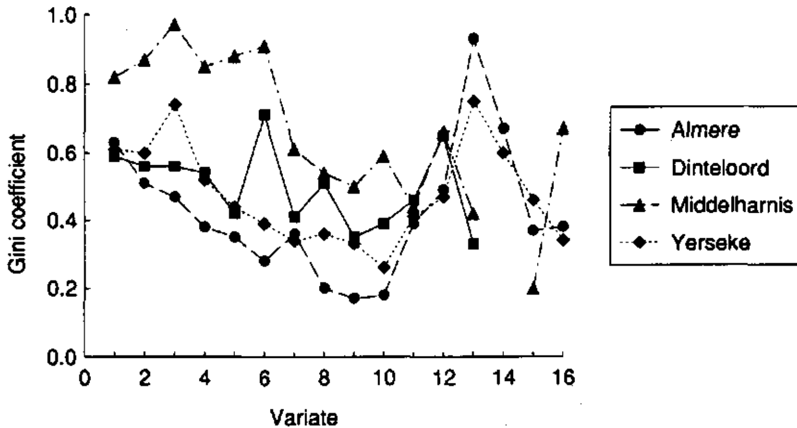

Figure 3.4 Values of Gini coefficient for 16 variates of *Puccinia punctiformis* populations at four sites in The Netherlands. Gini coefficient is missing for the variates 14-16 of Dinteloord, and for variate 14 of Middelharnis.

systemic infection (variates 1-6 and 13-14, Figure 3.4). The profile of the Gini values of the variates differed significantly between sites (Table 3.4B). The population at Middelharnis was most hierarchical, the one at Almere least. Differences between populations were smallest for the number of lesions on shoots with local lesion infection (variates 11 and 12, Figure 3.4). Ranking for hierarchy was inverse to the ranking for size of the population (Table 3.4).

Host populations

The profiles of the mean values of the six counting dates of *C. arvense* shoots differed significantly between sites (Table 3.5A, next page). The population at Dinteloord was the largest, and that at Middelharnis the smallest. At all sites, most values of the Gini coefficient were smaller than 0.5, indicating relatively little hierarchy (Table 3.5B). The profile of the Gini values of the six counting dates differed significantly between sites. The host population at Middelharnis was most hierarchical, and the one at Dinteloord least. The ranking for hierarchy was inverse to the ranking for size of the population (Table 3.5A, B).

Table 3.5 Size and spatial variation of *Cirsium arvense* populations at four sites in The Netherlands. Entries are mean number of shoots (A) and value of the Gini coefficient (B) based on 24 plots, of 4 m * 4 m each, per site. Sites ranked with Friedman's Test for Randomized Blocks.

Count	Almere	Dinteloord	Middelharnis	Yerseke
A. Mean				
June 1989	193	537	85	242
September 1989	487	730	99	411
October 1989	385	580	47	308
June 1990	386	540	53	357
September 1990	558	784	113	427
October 1990	391	313	72	430
Ranking of sites:	Dinteloord	3.67		
	Almere	2.83		
	Yerseke	2.50		
	Middelharnis	1.00		
n = 6	$X^2 = 13.4$		$P < 0.01$	
B. Gini coefficient				
June 1989	0.30	0.13	0.43	0.43
September 1989	0.19	0.13	0.43	0.34
October 1989	0.20	0.13	0.51	0.36
June 1990	0.15	0.12	0.49	0.32
September 1990	0.14	0.11	0.49	0.31
October 1990	0.17	0.18	0.59	0.25
Ranking of sites:	Middelharnis	4.00		
	Yerseke	3.00		
	Almere	1.83		
	Dinteloord	1.17		
n = 6	$X^2 = 17.0$		$P < 0.001$	

DISCUSSION

Size of the *P. punctiformis* populations was inversely related to hierarchy expressed by the Gini coefficient. The more the *P. punctiformis* populations were hierarchical, the more the pathogen was restricted to a few shoots of the host or a few locations at a site. In general, three factors might contribute to a spatially unequal distribution of pathogens (Burdon *et al.*, 1989): the pattern of pathogen dispersal, variation in the physical environment, and variation in host resistance. These three factors are discussed here to explain the strong hierarchical spatial structure of the *P. punctiformis* populations at the sites Dinteloord and Middelharnis.

At Middelharnis, the host population was small and concentrated in a few plots. Dispersal of *P. punctiformis* within the host population might have been hampered by the relatively few and unequally distributed shoots of the host, and this hampering might thus explain the strong hierarchy of the *P. punctiformis* population. In contrast, the host population at Dinteloord was relatively large and equally distributed. Poor dispersal of *P. punctiformis* within the host population seems an invalid explanation for the strong hierarchy of the *P. punctiformis* population at this site. There was no visible evidence for major variation of the physical environment. A better explanation seems variation of host resistance. Due to the recent colonization of this site by *C. arvense*, many host genotypes might be present. Evidence for such a colonization effect on the number of genotypes, is provided by Kik *et al.* (1990) for the clonal perennial *Agrostis stolonifera* L. A relatively large number of genotypes might have resulted in more variation of resistance at Dinteloord than at other sites.

Except for Middelharnis, hierarchy of *P. punctiformis* populations differed clearly according to phase of infection. Systemic infection showed more hierarchy than local lesion infection. Systemic infection is more damaging to *C. arvense* shoots than local lesion infection (e.g. Bailiss & Wilson, 1967). The question arises whether the degree of hierarchy is directly related to the extent of the damage that the pathogen causes, an ecological and phytopathological puzzling question.

In the present study, small pathogen populations were more hierarchical than large populations. Because of hierarchy, a small pathogen population might have a larger effect on the host population than would be expected from its size. Relatively few shoots of the host population are far more exposed to the pathogen than most other shoots. Since rust infection may reduce the ability of host plants to compete with other plants (Paul, 1989; Paul & Ayres, 1987b, 1990), an unequal exposure to *P. punctiformis* would result in a selective reduction of the competetive ability of *C. arvense* shoots. Selective reduction of the abilility of the host to compete might offer the opportunity for plants of other species to penetrate the host area at a few locations. Such first penetrations might be the bridgeheads for a further penetration of non-host plants into the host area. If so, consideration of hierarchy of pathogen populations provides a fresh view on the role of pathogens in plant communities.

CHAPTER 4

The role of clonal growth in the pathosystem *Cirsium arvense - Puccinia punctiformis*

SUMMARY

The impact of the pathogenic rust fungus *Puccinia punctiformis* on the population dynamics of the clonal host plant *Cirsium arvense* was studied using field data and a matrix population model. To account for the spatial growth pattern of *C. arvense*, the study area was divided into cells of 10 cm by 10 cm. The cells were classified as empty (no *C. arvense* shoots), diseased (shoots infected by *P. punctiformis*) or healthy. The spatial growth pattern of *C. arvense* could be described as 'static' or 'dynamic'. Abundance of *C. arvense* depended largely on 'dynamic' clonal growth. Shoots produced by 'dynamic' clonal growth had a lower probability to be infected by *P. punctiformis* than shoots produced by 'static' clonal growth. 'Dynamic' clonal growth seemed to serve as a mechanism of disease escape. The results are discussed with respect to the use of *P. punctiformis* as biological agent for control of *C. arvense* in grasslands.

INTRODUCTION

The role of pathogens in plant communities is subject of a continuing debate (Harper, 1977; Dinoor & Eshed, 1984; Zadoks, 1987; Burdon & Leather, 1990). Interactions of organisms within biocoenoses are complex, and experimental proof of the significance of pathogens is rather difficult. Studies of biological weed control have deepened the knowledge about pathogens in natural biocoenoses (Zadoks, 1987; Burdon, 1987; Harper, 1990). The reverse is also true, knowledge about the role of pathogens in plant communities may be used to develop methods of biological weed control (Dinoor & Eshed, 1984).

Pathogens may have a negative impact on wild host plants by reducing seed production (Paul & Ayres, 1986b; De Nooij & Van der Aa, 1987; Hasan & Aracil, 1991), reducing survival (Paul & Ayres, 1986a, 1987a; Hasan & Aracil, 1991), and reducing the ability

of interspecific competition (Paul, 1989; Paul & Ayres, 1987b, 1990). To use pathogens for biological weed control, the negative impact on individual host plants has to result in changes of population dynamics of the host.

Matrix population models were developed for demographic studies of human populations (Leslie, 1945, 1948). In the 1970's matrix population models were adopted by ecologists to study dynamics of plant populations (Caswell, 1989). In the original form of the models, individuals of a population were classified by age (Leslie, 1945, 1948). Other categories such as size (Lefkovitch, 1965) or combinations of categories (Goodman, 1969) can also be used. Incorporating plant disease, caused by pathogens, in matrix population models offers the possibility to estimate the role of disease in population dynamics of the host (Alexander, 1982).

Individuals of clonal plant species, the genets, consist of many genetically identical units, the ramets (Kays & Harper, 1974). Genets have characteristics with respect to distance and duration of inter-ramet connections. The resulting clonal growth pattern may spread the risk of mortality over the ramets belonging to a genet (Eriksson & Jerling, 1990). Individual ramets may die, but the genet persists. Persistence by way of vegetative reproduction is one of the properties of some troublesome weeds (Leackey, 1981).

The clonal plant species *Cirsium arvense* (L.) Scop. is the subject of the present study. Undesirable side effects of chemical herbicides have promoted interest in biological control of this weed. The rust fungus *Puccinia punctiformis* (Str.) Röhl. is considered as a biological agent to control *C. arvense* since a long time (e.g. Cockayne, 1915). Until now, no study was addressed to the impact, or potential impact, of *P. punctiformis* on population dynamics of *C. arvense*. Aim of the present study was to determine the impact of *P. punctiformis* on population dynamics of *C. arvense* in grasslands, and to evaluate the potential of *P. punctiformis* as a biological agent to control *C. arvense*.

MATERIALS AND METHODS

Study organisms

Cirsium arvense (L.) Scop. is a common perennial herb of grasslands. Aerial shoots continue as vertical subterranean shoots with scale leaves (caudices) in the soil (Leackey, 1981). The underground system consists further of horizontal and vertical roots. The horizontal roots are called creeping roots. A ramet consists of an aerial shoot and the underground parts belonging to it. New aerial shoots are produced on caudices and creeping roots (Sagar & Rawson, 1964). Production of new shoots depends, among other factors, on water soluble carbohydrates, which are transported from the aerial

shoots to the underground system (e.g. Otzen & Koridon, 1970). In The Netherlands, shoots emerge first above soil in March-April, and aerial shoots die before December. Establishment of seedlings in grasslands is rare (Amor & Harris, 1975) and persistence depends largely on clonal growth.

Shoots of *C. arvense* can be systemically infected by the rust fungus *Puccinia punctiformis*. Systemically infected shoots are chlorotic, have a reduced leaf area (Bailiss & Wilson, 1967), and their respiration rate is increased (Kourssanow, 1928). A second type of infection caused by *P. punctiformis*, local lesion infection, far less damaging to shoots than systemic infection (e.g. Bailiss & Wilson, 1967), is not considered in the present study.

Field data

Two grassland sites in The Netherlands were used. Distance between sites was about 100 km. Both sites were mown at the end of June before seed set, a common practice in The Netherlands to prevent dispersal of *C. arvense* seeds to farmers' land. The soil texture of both sites was clay-loam. The soil of the site Huissen had a higher content of nutrients, which may reflect its use as a meadow before starting the present study. The vegetation of the site Kemphaan (in the vicinity of the town of Almere) was dominated by *Festuca rubra* L., at the site Huissen by *Lolium perenne* L.

At each site, 24 plots of 1 m by 1 m were laid out in a 3 by 8 pattern. Distance between plots was 4 m. At the time of census, a grid of 10 by 10 cells was placed over a plot. The state of each cell was classified as diseased (at least one systemically infected *C. arvense* shoot), healthy (no systemically infected *C. arvense* shoot) or empty (no *C. arvense* shoot). Dead shoots were not considered. Censuses were in May, June (before mowing), August and September in the preceding year, and May of the following year, in the period 1990-1991 at Kemphaan and in the period 1991-1992 at Huissen. Transition probabilities were determined as the fraction of cells in one state at the preceding census having one of the three states at the following census. This resulted in nine probabilities of transition between two times of census. Because of the relatively low number of diseased cells, data of all 2400 cells at a site were pooled to determine the transition probabilities. Where required, transition probabilities were statistically tested. A frequency analysis based on the G-statistic was used (Sokal & Rohlf, 1981).

The model

The transition probabilities were ordered in 3 by 3 projection matrices, and were used in a matrix population model (Caswell, 1989):

$$\mathbf{n}_{t+1} = \mathbf{A}\mathbf{n}_t$$

Here, **n** is a vector of the distribution of the cells over the three states at the preceding time of census (t), and the following time of census (t+1), respectively. The projection matrix **A** represents the nine possible transitions between the three states of the cells from time t to time t+1.
The use of matrix population models in the present study was deviant from the common use of this type of models. First, cells were used as 'population' units in stead of shoots. Second, the number of units was fixed (2400 per site), i.e. the 'population' of cells could not increase or decrease (the eigenvalue λ was always 1).
Projection matrices of the transitions May-June, June-August, August-September and September-May were used in the model to represent one year (May-May). Starting with the classification of cells in May of the first year, the model was run several years until a stable distribution of cells over the three states had established in May.

RESULTS

Field data

At both sites, the number of diseased cells was low, compared to the number of healthy cells, at the beginning of the study in May 1990 and 1991, respectively (Table 4.1). The mean density of *C. arvense* was between one and two shoots per healthy or diseased cell. The greater part of the cells was empty. Most or all of the nine possible transitions between the three states of the cells were determined in the four periods between the preceding and the following May (Table 4.2). At both sites, the probability that cells (healthy or diseased) became empty was highest in the period September-May (first and second column, third row). In all periods, except the period June-August at Kemphaan, diseased cells had a higher probability to become empty than

Table 4.1 Distribution of cells over the three states diseased, healthy and empty at two sites in The Netherlands. At the site Kemphaan, in May 1990, and at the site Huissen in May 1991, both at the beginning of the study.

Site	Diseased	Healthy	Empty
Kemphaan	62	426	1912
Huissen	29	380	1991

Table 4.2 Transition probabilities of cells between the states diseased, healthy and empty in four periods at two sites ordered as projection matrices. Columns represent the state of the cells at the preceding census, rows represent the state of the cells at the following census.

Site	Period		Diseased	Healthy	Empty
Kemphaan	May-June	Diseased	0.274	0.012	0.005
		Healthy	0.290	0.650	0.057
		Empty	0.436	0.338	0.938
	June-August		0.344	0.030	0.005
			0.250	0.495	0.096
			0.406	0.475	0.899
	August-September		0.182	0.013	0.003
			0.273	0.768	0.032
			0.545	0.219	0.965
	September-May		0.000	0.005	0.001
			0.125	0.197	0.057
			0.875	0.798	0.942
Huissen	May-June		0.655	0.026	0.001
			0.000	0.774	0.032
			0.345	0.200	0.967
	June-August		0.267	0.017	0.004
			0.233	0.603	0.073
			0.500	0.380	0.923
	August-September		0.045	0.000	0.000
			0.182	0.808	0.012
			0.773	0.192	0.988
	September-May		0.000	0.040	0.006
			0.000	0.285	0.054
			1.000[a]	0.675	0.940

[a] based on 1 cell only

healthy cells. The difference between diseased and healthy cells was largest in the period August-September. The probability to become empty in September depended significantly ($P < 0.001$) on the state of the cell (diseased or healthy) in August. The adjusted G-values were 14.7 and 31.0 at Kemphaan and Huissen, respectively (1 *df*). Cells that were occupied by *C. arvense* shoots in May of the following year had a higher probability to be diseased if they were healthy in the preceding May than if they were empty (Table 4.3, next page). The adjusted G-values were 6.2 and 4.1 at

Table 4.3 The number of cells diseased in May of the following year in relation to the state of the cells in May of the preceding year, at two sites in The Netherlands.

Site	State of the cells in 1990	Total number of cells occupied by *C. arvense* in 1991	State of the cells in 1991	
			Diseased	Healthy
Kemphaan	Healthy	55	3	52
	Empty	134	0	134

	State of the cells in 1991	Total number of cells occupied by *C. arvense* in 1992	State of the cells in 1992	
			Diseased	Healthy
Huissen	Healthy	92	15	77
	Empty	133	10	123

Kemphaan and Huissen, respectively (1 *df*, $P < 0.05$). The number of cells diseased in the preceding May, and occupied by *C. arvense* shoots in the following May, was too low (5 at each site) to be included in the statistical analysis.

Model analysis

The projection matrices presented in Table 4.2 were used in the matrix population model. The resulting stable state distribution for May and the computed projection matrix over a year (May-May), represent the population dynamics of *C. arvense* (Figure 4.1). The greater fraction of cells occupied by *C. arvense* shoots in the preceding May will be empty in the following May irrespective their state (diseased or healthy) in the preceding May. A small fraction of cells (10-15 %) will be occupied by *C. arvense* shoots in both the preceding and the following May. Clonal growth in these cells is 'static'. About 7 % of cells empty in the preceding May are occupied by shoots in the following May, representing the 'dynamic' clonal growth. The 'dynamic' component of clonal growth will contribute most to the total number of occupied cells, e.g. about $(0.074*2213) + (0.001*2213) = 166$ of the total 187 cells at Kemphaan. The number of cells occupied by *C. arvense* is, therefore, most sensitive to the transitions empty-healthy and empty-diseased.

Figure 4.1 indicates that a shift from the transition empty-healthy towards the transition empty-diseased will result in a relatively large increase of the number of diseased cells. For example Kemphaan, the transition empty-healthy set from 0.074 to zero and the

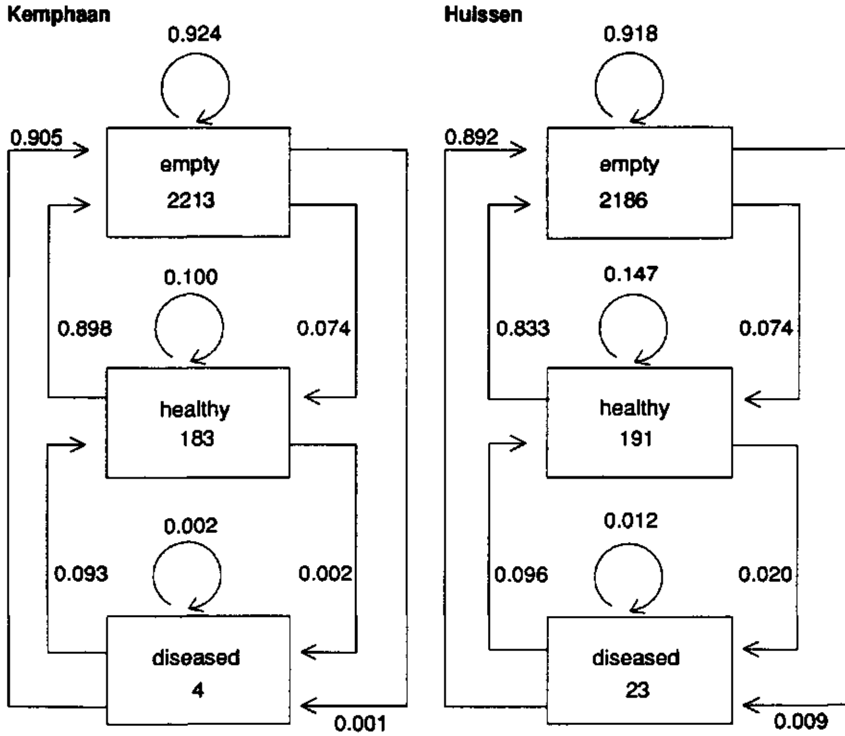

Figure 4.1 Diagram of population dynamics of *Cirsium arvense* at two sites in The Netherlands. Entries of the boxes are the numbers of cells according to the stable state distribution in May, computed with a matrix population model. Arrows indicate the transition probabilities between the three states from May in the preceding year to May in the following year.

transition empty-diseased set at 0.074 + 0.001 = 0.075 will result in about 166 diseased cells and 21 healthy cells. The projection matrix over a year (May-May), used in the diagram, was computed from the projection matrices of the periods May-June, June-August, August-September and September-May. The same manipulation, the fraction of cells of the transition empty-healthy added to the fraction of cells of the transition

Table 4.4 Stable state distribution in May after manipulation of the projection matrices of May-June, June-August, August-September and September-May, respectively, at two sites. The way of manipulation is explained in the text.

Site	Period	Diseased	Healthy	Empty
Kemphaan	May-June	4	182	2214
	June-August	3	171	2226
	August-September	3	179	2218
	September-May	121	65	2214
Huissen	May-June	23	187	2190
	June-August	20	166	2214
	August-September	22	183	2195
	September-May	139	68	2193

empty-diseased, was conducted in each of these matrices separately to estimate the most sensitive period. Only the manipulation in the period September-May resulted in a stable state distribution in May with a higher number of diseased cells than healthy cells (Table 4.4).

DISCUSSION

Diseased cells emptied faster than healthy cells. A high number of diseased cells seems a hazard to persistence of *C. arvense*. An early death of systemically infected shoots, compared to healthy shoots, was already reported by Watson and Keogh (1980). The relatively fast mortality of systemically infected shoots may be caused by drought-stress as Paul and Ayres (1984) demonstrated for plants of *Senecio vulgaris* L. infected by the rust fungus *Puccinia lagenophorae* Cooke. The epidermis of a systemically infected *C. arvense* shoot is seriously ruptured by the development of *P. punctiformis* pustules, and drought-stress is obvious.

At both sites, the number of diseased cells was low compared to the number of healthy cells. The data indicated that the pattern of clonal growth may be a factor reducing

disease incidence. Cells occupied by *C. arvense* shoots in both the preceding and the following May ('static' clonal growth) had a higher probability to be diseased in the following May than cells which were empty in the preceding May and occupied in the following May ('dynamic' clonal growth). 'Dynamic' clonal growth served as a mechanism of disease escape. Disease escape in relation to the pattern of clonal growth has also been determined for the clonal plant *Lactuca siberica* L. and its pathogen the rust fungus *Puccinia minussensis* Thum. (Wennström & Ericson, 1992). The escape mechanism was studied more extensively for some tropical trees (see review of Augspurger, 1990). These studies demonstrate the complexity of the escape mechanism. The escape mechanism as found in the present study, is rather difficult to explain without additional data. One explanation might be a relative lack of infectious material of *P. punctiformis* at the localities newly colonized by way of the creeping roots. If so, for biological control of *C. arvense*, *P. punctiformis* has to be applied to the whole area invaded by the creeping roots of *C. arvense* to prevent disease escape.

To achieve an early control of *C. arvense* within the growing season, disease incidence should be as high as possible in May. The results of the model indicated that the transitions in the period September-May should be manipulated to have a relatively high disease incidence in May. Because root buds of *C. arvense* produced in late summer and autumn develop to the new aerial shoots in spring (Sagar & Rawson, 1964), infection of these buds might result in systemically infected shoots in spring. In experiments under controlled conditions, application of infectious material of *P. punctiformis* onto root pieces of *C. arvense* resulted in systemically infected shoots (Van den Ende *et al.* 1987; French & Lightfield, 1990). Provided a similar application can be realized in the field, a relatively high fraction of diseased cells in the spring seems achievable.

An increase of systemic infection incidence in the spring is just one of the aspects of biological control. The 'dynamic' clonal growth was a major feature of the *C. arvense* life history at the sites studied. Unknown is the relation between systemic infection and the ability of 'dynamic' clonal growth. It is obvious that translocation of carbohydrates from systemically infected shoots to the root system is reduced. Because several shoots may be attached to the same root system, losses due to systemically infected shoots might be compensated by uninfected shoots attached to the same root system. If such compensation occurs, the question arises which fraction of *C. arvense* shoots should be systemically infected before losses are no longer compensated by healthy shoots, and 'dynamic' clonal growth will be inhibited.

The demographic study revealed a major feature of the life-history of *C. arvense*, 'dynamic' clonal growth, the function of this feature as a mechanism of disease escape, and the lack of knowledge about the impact of *P. punctiformis* on this major component. Demographic studies seem to be appropriate tools to improve the screening of pathogens and their subsequent development of pathogens as agents for biological weed control.

CHAPTER 5

The effect of temperature on the germination of teliospores of *Puccinia punctiformis*

SUMMARY

Germination of teliospores was analysed as a time-dependent process. Non-linear regression was used to fit germination progress curves for 5, 10, 15 and 20 °C. Germination was characterized by the maximum fraction of teliospores germinated, the mid germination time (half of the maximum germinated) and the rate of germination at the mid germination time. Temperature had a major effect on the mid germination time and, to a smaller extent, on the rate of germination. The mid germination time was shortest at 15 °C, longer at 10 and 20 °C, and longest at 5 °C. The rate of germination was highest at 10 and 15 °C, and lower at 5 and 20 °C. No effect of temperature was detected on the maximum fraction of teliospores germinated. The consequences of the germination dynamics for infection of *Cirsium arvense* root buds by *P. punctiformis* are discussed.

Puccinia punctiformis (Str.) Röhl. infects *Cirsium arvense* (L.) Scop. systemically by means of teliospores (French & Lightfield, 1990; Van den Ende *et al.*, 1987). Teliospores are considered as a potential bioherbicide for *C. arvense* (French & Lightfield; 1990, Turner *et al.*, 1986; Van den Ende *et al.*, 1987). Current research demonstrated that root buds of *C. arvense* act as ports of entry for the rust (Chapter 6). Root buds of *C. arvense* develop in the range of 5-30 °C (Özer, 1969). To evaluate the practical use of teliospores as a bioherbicide, knowledge of the influence of temperature on root bud infection is required. Quantitative data on germination are necessary to conduct infection experiments adequately.

Germination of *P. punctiformis* teliospores is stimulated by volatile extracts from *C. arvense* roots (French *et al.*, 1988). Frantzen (unpublished) demonstrated that instead of extracts of the roots, root pieces may be used to stimulate germination.

French & Lightfield (1990) reported that only a low fraction of teliospores germinated at temperatures below 11 °C or above 24 °C after 7 days incubation. They did not consider the dynamic component of germination, i.e. the change of the fraction of

teliospores germinated in time. The present study was designed to determine the influence of temperature on the dynamics of germination.

MATERIALS AND METHODS

General

An isolate of *P. punctiformis* was collected from infected *C. arvense* shoots in a grassland in the vicinity of the town of Nijmegen (Ooy) and cultured on a *C. arvense* clone from the same site in a climate room. Root pieces were inoculated with spores and the resulting systemically infected shoots were, following Waters (1928), placed at 10-13 °C and low light intensity (< 5 W m^{-2}) to stimulate the production of teliospores. Spore samples were collected with a cyclone spore collector and stored for 2-4 months in a refrigerator (about 5 °C) at 45% RH. Teliospores were rehydrated at 80% RH during at least 24 h before they were used in the experiments.
Teliospores were evenly deposited on slides by means of a settling tower. The slides had been covered previously with 1% water agar (prepared with distilled water). Each slide was placed in a separate Petri dish with wetted blotting paper on the bottom. Three or four root pieces of *C. arvense* (total weight 0.6-0.7 g) were placed next to a slide to stimulate germination of teliospores by means of volatile components of the roots. The Petri dishes were placed in the dark at various temperatures (see below) in thermostats. Deviations from the chosen temperatures were within the range of ±1 °C. After incubation, the fraction of teliospores germinated was determined by observing at least 150 teliospores per slide. Teliospores were considered to be germinated if a metabasidium had been formed by one or both of the telial cells.

Temperature selection

In a preliminary experiment, the range of temperature to be tested was selected. Teliospores, collected from one systemically infected shoot on one date, were dispersed over 33 slides. The slides were randomly assigned to an incubation temperature of 5, 10, 15, 20 or 25 °C. At 5, 10 and 15 °C, teliospores were incubated during 7, 14 or 21 days, at 15 and 20 °C, only during 7 days. For each temperature and incubation time, three slides (replicates) were used.

Germination dynamics

Based on the results of the preliminary experiment, the effect of temperature on the dynamics of germination was determined for 5, 10, 15 and 20 °C. Teliospores were dispersed over eight series of 15 slides each. For each series a subsample of a spore sample, collected from several systemically infected shoots and on several dates, was used. Two series were randomly assigned to each temperature and served as replicates. Because the two replicates of a temperature were placed together in the same thermostat, they were not considered as independent in the statistical analysis. Germination was determined after various times of incubation. For 15 and 20 °C, germination was determined at daily intervals until 14 days after the beginning of the incubation. For 10 °C, germination was determined at two-day intervals until 20 days after the beginning of the incubation, and for 5 °C at two-day intervals until 30 days. At each time of observation, germination of teliospores was determined on one slide for each temperature and replication. This experiment (referred to as experiment I) was repeated in the experiments II and III. The spore sample used in experiment I was also utilised in the experiments II and III. In each of the experiments, temperature was randomly assigned to the thermostats.

Data analysis

Data analysis of the experiments I-III was conducted in two steps. First, curves were fitted to the data by non-linear regression using Genstat (Payne & Layne, 1987). Log-logistic curves (Campbell & Madden, 1990) were fitted :

$$y = c * g \qquad (1)$$

and

$$g = 1/\{1+\exp(-b*\ln(t/\tau))\} \qquad (2)$$

where y is the fraction of teliospores germinated, c is the upper asymptote of y (the germinable fraction of teliospores), g is the function describing the germination of germinable teliospores, b is the shape parameter, t is the time in days and τ is the mid germination time, at which a fraction 0.5 of the germinable spores is germinated. To restrict values of c to the range 0-1, the parameter γ was estimated in the curve fitting routine and transformed into c using the equation:

$$c = 1/(1+\exp(-\gamma)) \qquad (3)$$

The curves were fitted using a binomial distribution with correction for over-dispersion (McCullagh & Nelder, 1989). The binomial distribution accounted for the differences in variance of germination depending on incubation time. Over-dispersion accounted

for factors affecting the independence of teliospores with respect to germination, i.e. deviations from the binomial distribution.
Curves were fitted to data of each temperature and replicate within an experiment. The rate of germination (dg/dt) at the mid germination time (τ) was computed:

$$v = b/(4*\tau) \tag{4}$$

For each temperature and experiment, the mean values of the parameters were computed from the values of the two replicates. These mean values were used in the subsequent statistical analysis, in which the three experiments were treated as blocks in a randomized complete block design with four temperature treatments.
The effect of temperature on the parameters was first tested by a distribution free permutation F-test (Gilligan, 1986) because, according to Ross (1981), parameter estimates need not to be exactly normally distributed. The results of the permutation test were compared with the results of an ANOVA. ANOVA was performed to test the temperature effect on linear and non-linear trends (Gilligan, 1986) providing additional information about the effect of temperature on the germination dynamics.

RESULTS

Temperature selection

Hardly any of the teliospores incubated at 25 °C had germinated after 21 days of incubation (Table 5.1). The fraction of teliospores germinated was about 0.65 or higher for teliospores incubated at 5, 10, 15 and 20 °C.

Table 5.1 The fraction of teliospores of *Puccinia punctiformis* germinated at various temperatures and incubation times in the preliminary experiment[a].

	Incubation time (days)					
Temperature (°C)	7	(SE)	14	(SE)	21	(SE)
5	0.00	(0.00)	0.01	(0.01)	0.66	(0.13)
10	<0.01	(<0.01)	0.79	(0.02)	0.84	(0.03)
15	0.78	(0.01)	-	-		
20	0.84	(0.03)	-	-		
25	0.00	(0.00)	0.01	(<0.01)	0.04	(0.01)

[a] Entries are means of three replicates.

Germination dynamics

The quality of fit of curves varied as reflected by the standard errors of the estimates of the parameters (Table 5.2). Estimates of the parameter b were less accurate than estimates of the parameters c and τ. Fitted curves for 20 °C in experiment I,

Table 5.2 Estimated parameters (and SE) of a log-logistic model[a] fitted to data on germination of *Puccinia punctiformis* teliospores, depending on temperature.

			Parameter		
Experiment	Temperature	Replicate	c	τ	b
I	5	1	0.64 (0.04)	20.0 (0.6)	13.0 (2.8)
		2	0.60 (0.02)	19.4 (0.3)	14.7 (1.6)
	10	1	0.70 (0.02)	9.0 (0.2)	8.7 (1.2)
		2	0.62 (0.06)	8.6 (0.7)	9.3 (4.5)
	15	1	0.72 (0.11)	6.9 (0.9)	3.8 (1.2)
		2	0.53 (0.05)	6.4 (0.6)	5.2 (1.5)
	20	1	0.72 (0.16)	9.5 (1.5)	3.6 (0.8)
		2	0.81 (0.21)	8.7 (1.8)	3.4 (1.1)
II	5	1	0.59 (0.06)	17.2 (0.9)	7.5 (1.9)
		2	0.56 (0.05)	16.9 (0.7)	8.5 (1.9)
	10	1	0.56 (0.06)	8.2 (0.6)	8.2 (3.2)
		2	0.55 (0.04)	7.6 (0.3)	14.6 (5.3)
	15	1	0.59 (0.05)	5.5 (0.4)	8.7 (3.4)
		2	0.66 (0.02)	5.4 (0.1)	9.2 (1.1)
	20	1	0.69 (0.14)	6.8 (1.3)	3.3 (1.3)
		2	0.37 (0.06)	5.2 (0.9)	5.5 (3.0)
III	5	1	0.44 (0.05)	21.6 (0.7)	10.8 (1.7)
		2	0.55 (0.10)	22.7 (1.2)	9.4 (1.7)
	10	1	0.56 (0.10)	10.6 (1.1)	4.9 (1.5)
		2	0.55 (0.03)	8.3 (0.2)	21.8 (8.6)
	15	1	0.48 (0.05)	6.1 (0.4)	7.9 (2.9)
		2	0.44 (0.03)	5.4 (0.3)	12.0 (4.4)
	20	1	0.50 (0.06)	6.0 (0.6)	6.3 (2.3)
		2	0.37 (0.06)	5.7 (0.9)	5.8 (3.3)

[a] $y = c * g$ and $g = 1/\{1+\exp(-b*\ln(t/\tau))\}$ in which y is the fraction of teliospores germinated, c is the germinable fraction of teliospores, g the function describing the germination of germinable teliospores, b is the shape parameter, t is the time in days, and τ is the mid germination time at which y is $0.5 * c$.

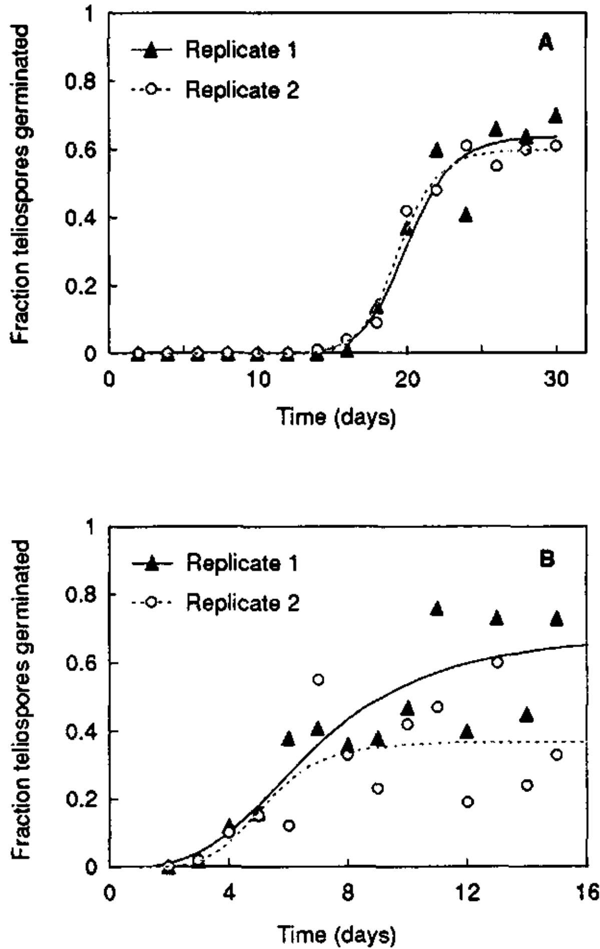

Figure 5.1 Two examples of fitting curves to data of germination of *Puccinia punctiformis* teliospores. Germination at 5 °C in experiment I (A) and at 20 °C in experiment II (B). Entries are observed fractions, and lines were fitted to the data using a log-logistic model. The parameter estimates of the log-logistic model are presented in Table 5.2.

replicate 2, and experiment II, replicate 1, had relatively large errors for the estimates of all three parameters. Examples of curves with better and poorer fit, respectively, are presented in Figure 5.1.

The significance levels of the effect of temperature on germination determined by ANOVA differed only slightly from those of the permutation test (Table 5.3). Analysis of variance was, therefore, used to test the temperature effect on linear and non-linear trends. No effect of temperature could be detected on parameter c. Parameter τ depended both linearly and non-linearly on temperature: the value of τ differed significantly between lower and higher temperature, and suggested a trough around 15 °C. Parameter b depended linearly on temperature: the value of b differed significantly between lower and higher temperature. Parameter v depended non-linearly on temperature: the value of v showed a hump at 10 - 15 °C.
Substitution of the parameter estimates in equations (1) and (2) resulted in curves with a delay of germination at 5 and 10 °C, and relatively high rates of germination at 10 and 15 °C (Figure 5.2, next page). At 20 °C, the maximum level is reached late relative to the early onset of germination, reflecting the relative low v.

Table 5.3 Effects of temperature on parameters of a log-logistic model[1] fitted to data of germination of *P. punctiformis* teliospores in the experiments I-III.

	Parameter[2]			
Temperature	c	τ	b	v[3]
5 °C	0.57	19.7	10.6	0.14
10 °C	0.59	8.7	11.2	0.34
15 °C	0.57	6.0	7.8	0.34
20 °C	0.58	7.0	4.7	0.18
P (permutation)[4]	0.98	0.009	0.064	0.051
P (ANOVA)[4]	0.98	<0.001	0.058	0.035
- linear	0.95	<0.001	0.016	0.490
- non-linear	0.91	0.001	0.400	0.019

1 $y = c * g$ and $g = 1/\{1+\exp(-b*\ln(t/\tau))\}$ in which y is the fraction of teliospores germinated, c is the maximum fraction of teliospores germinated, g is the function describing the germination of germinable teliospores, b is a shape parameter, t is the time in days, and τ is the mid germination time at which $y = 0.5 * c$.

2 Entries are the means of three estimates of the parameters, one estimate per experiment.

3 The rate of germination (dg/dt) at the mid germination time: $v = b / (4 * \tau)$.

4 Effect of temperature tested using a permutation test and ANOVA. The temperature effect was split in linear and non-linear trends in ANOVA.

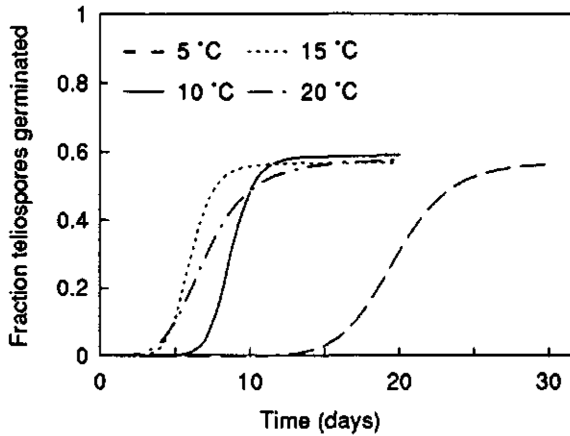

Figure 5.2. Germination of teliospores of *Puccinia punctiformis*. Germination progress curves calculated with the parameter estimates of Table 5.3.

DISCUSSION

The temperature optimum curve for germination of teliospores of *P. punctiformis* reported by French and Lightfield (1990) was based on data obtained after 7 days of incubation. Germination after 7 days of incubation in the preliminary experiment (Table 5.1) corresponded well with the optimum curve of French and Lightfield (1990). Incubation of teliospores during 14 and 21 days at 10 and 5 °C, respectively, resulted in fractions of teliospores germinated that approximated those obtained at 15 and 20 °C during 7 days. The result indicated a time dependency of germination. At 25 °C, the fraction of teliospores germinated was still low after 21 days incubation. Incubation time seemed to have relatively little influence on germination at this temperature. Therefore, 25 °C was not included in the experiments I-III.

The results of the experiments I-III confirmed that time is a major factor influencing germination of fungal spores as already shown by other studies (e.g. Eisensmith *et al.*, 1985). The log-logistic model used to describe the germination dynamics was applied to all data. Some curves had a low fit, especially at 20 °C, and, another model might have been applied in those cases. To compare data of the various temperatures and experiments, however, the same model was applied to all data.

The model used was chosen because of the biological meaning of the parameters. The parameter c indicates the germinable fraction of teliospores. In the present study, the parameter had, independently of temperature, a mean value about 0.6. This intermediate value, on a scale of 0-1, reflects mixing several spore samples into one large sample. Variation in the conditions of the climate room or genetic variation within the *P. punctiformis* isolate probably caused differences in ripening or vitality of teliospores sampled from various shoots and on various dates. Heterogeneity of germination in the present study agrees with the heterogeneity of germination reported for *P. punctiformis* teliospores sampled from the field (Turner *et al.*, 1986).
The function g describes the germination dynamics of the germinable teliospores. The parameter τ of this function indicates the time at which one half of the germinable teliospores has germinated, the mid germination time. The major effect of temperature was on this parameter. Germination of teliospores was strongly delayed at 5 °C. Experiments directed toward infection of *C. arvense* root buds by *P. punctiformis* have to take this temperature effect into account. At low temperature, infection has to be expected at a much later time than at higher temperature.
The shape parameter b has biological meaning because it determines, together with τ, the rate of germination at the mid germination time. The rate of germination showed a non-linear trend with relatively high values at intermediate temperature (10, 15 °C) and relatively low values at the extremes (5, 20 °C). These results indicate an optimum for the rate of germination, which may result in a higher infection rate (more infections per unit time) around 10-15 °C than around 5 and 20 °C.
The results of the present study suggest that the germination characteristics of teliospores allow biological control of *C. arvense* by *P. punctiformis* in the range 5-20 °C. Infection experiments are required to conclude which temperature will be best for biological control. The germination dynamics described here, provide a basis to conduct the infection experiments.

CHAPTER 6

Quantitative resistance of *Cirsium arvense* to root bud infection by *Puccinia punctiformis*

SUMMARY

A method is presented to test clones of *Cirsium arvense* for resistance to root bud infection by the rust fungus *Puccinia punctiformis*. Root buds were stained and cleared to detect mycelium of the rust. The fraction of root buds infected was determined and used as a measure of resistance. Clones of *C. arvense*, collected from three sites, were tested for resistance. Variation in resistance was determined between and within sites. The results are discussed with a view to using *P. punctiformis* to control *C. arvense*.

INTRODUCTION

The interest in using fungi for biological weed control increases, and more knowledge is required about fungi on wild plants, in general, and on weeds in special (Hasan & Ayres, 1990). The rust *Puccinia punctiformis* (Str.) Röhl. is one of the fungi that may be developed into a bioherbicide, provided that more knowledge is available about the biology of the rust and its host *Cirsium arvense* (L.) Scop.

The clonal plant *C. arvense* is a troublesome weed (Donald, 1990) on which *P. punctiformis* parasitizes by systemic and local lesion infection. Compared to uninfected shoots and shoots with local lesions, systemically infected shoots show large growth disturbances (Bailiss & Wilson, 1967). In recent years, research on biological control of *C. arvense* is aimed at the increase of the incidence of systemic infection by way of inoculating root buds with teliospores (French & Lightfield, 1990; French, 1991).

Resistance of plants to fungal infection is one of the factors inhibiting growth and development of fungi, and has been reported for wild plants (reviewed in Fritz &

Simms, 1992). Resistance might, therefore, limit the use of fungi for biological weed control. Here, we present a method to test *C. arvense* clones for resistance to root bud infection by *P. punctiformis* and results of resistance tests.

MATERIALS AND METHODS

Plant and rust cultures

Clones of *C. arvense* were cultured by cutting roots into pieces of 3-10 cm length and placing the root pieces in pots filled with commercial potting soil (six parts peat to one part sand). After three to four months, roots of the potted plants were again cut into pieces of 3-10 cm and potted.

A clone, sampled from the site Ooy, was maintained at the institute since autumn 1987. In the summer of 1991, *C. arvense* roots were collected at two other sites, Almere and Dinteloord. A root piece was sampled at each sixth meter along a straight line. In total, eight root pieces were sampled at each site. The eight root pieces were cultured as distinct clones. Some of the collected clones died in the greenhouse. From the remaining ones, three clones of each site were selected for the subsequent resistance tests. Selection was based on the distance between the clones at the sites (see table 6.2 of results). Before the tests started, the six newly selected clones and the clone Ooy were grown in a climate room at 20 °C, 60-70 % RH and 14 hours light (35-45 W m^{-2}) per day during at least two months.

In the autumn of 1987, an isolate of *P. punctiformis* was collected from *C. arvense* shoots with local lesions at the site Ooy (isolate Ooy). The rust was cultured by inoculating root pieces of the clone Ooy with a mixture of teliospores and urediniospores and collecting spores from the resulting systemically infected shoots. Production of teliospores was induced by placing systemically infected shoots at low light intensity (< 5 W m^{-2}) and low temperature (10-13 °C) during about one week, a routine similar to the one Waters (1928) used to induce teliospore production on shoots with local lesions. Spore mixtures of more than 40 % teliospores were stored in a refrigerator (4-5 °C) at 45% RH. In the autumn of 1990, a second isolate of the rust was collected from shoots with local lesions at the site Almere (isolate Almere). The isolate was stored until the summer of 1992 and then multiplied as mentioned above. One of the clones from the Almere site (Almere 6) was used to multiply the isolate.

Resistance tests

Inoculation. Roots of clones were cut into pieces of 2 cm length, and root pieces were selected on diameter (0.2 - 0.4 cm) and absence of (visible) buds. After rinsing the root pieces in de-ionized water, they were placed on wetted blotting paper in Petri dishes (diameter 11 cm). Ten root pieces per clone were placed in one Petri dish. Root pieces were inoculated with *P. punctiformis* spores using a settling tower to achieve an even distribution. The spore samples contained 70-80% teliospores and 20-30% urediniospores. Germination experiments (data not presented) indicated that germination of urediniospores was < 1% so that infection of root buds by way of urediniospores is unlikely, if possible at all. The mean density of deposited teliospores was about 1700 cm^{-2}. After inoculation, Petri dishes were closed, wrapped in aluminium foil and placed at 10 or 15 °C during three weeks.

Root bud infection. After the three weeks of incubation, root buds were counted, the length of each root bud was measured and each root bud was screened for infection (see below). Root buds were divided in two length classes: < 0.8 cm and ≥ 0.8 cm. The method to detect mycelium of *P. punctiformis* in the root buds is based on a method of Bruzzese and Hasan (1983) to detect mycelium of rust fungi in leaves. Root pieces, bearing buds, were placed in a staining solution. The staining solution consisted of: 300 ml ethanol, 150 ml distilled water, 125 ml lactic acid (90%), 150 g phenol, 450 g chloralhydrate and 0.6 g trypan blue. After 48 hours of staining, root buds were cleared during 24 hours in a clearing solution (250 g chloralhydrate in 1000 ml distilled water). After clearing, root buds were rinsed in distilled water and separated from the roots. The leaf primordia of a root bud were dissected one by one, placed on microscope slides and mounted in a permanent polyvinyl alcohol medium (Omar *et al.*, 1978). The leaf primordia were screened for rust mycelium using a magnification of 160X.

Experimental design. Experiments were conducted in two periods, February-June 1992 (experiment I) and November-December 1992 (experiment II). The isolate Ooy was used in experiment I, and the isolate Almere in experiment II. Within each experiment, root pieces were inoculated at six different dates. At each date 10 root pieces (one Petri dish) were inoculated per clone and temperature. The data of the six inoculation dates of an experiment were pooled to achieve appropriate numbers for statistical analysis. First, data were analysed by hierarchical log-linear models, based on the G-statistic (Sokal & Rohlf, 1981) and computed with SPSS/PC+ (Norusis, 1990). Modelling started with a saturated model containing all interactions, i.e. all variance of the data was explained ($G = 0$, $P = 1$). To select the most relevant interactions, explaining a

substantial part of the variance, interactions were removed one by one from the model and the resulting increase of the G-value was computed. If the removal of an interaction resulted in a significant increase of G ($P < 0.05$), the interaction was maintained in the model. Tested variables were clone (seven clones), temperature (10, 15 °C), length of root buds (two classes: < 0.8 cm and ≥ 0.8 cm) and infection of root buds (absence/presence of mycelium). Subsequently, likelihood ratio tests, based on the G-statistic, were used to test for separate two-way interactions (Sokal & Rohlf, 1981).

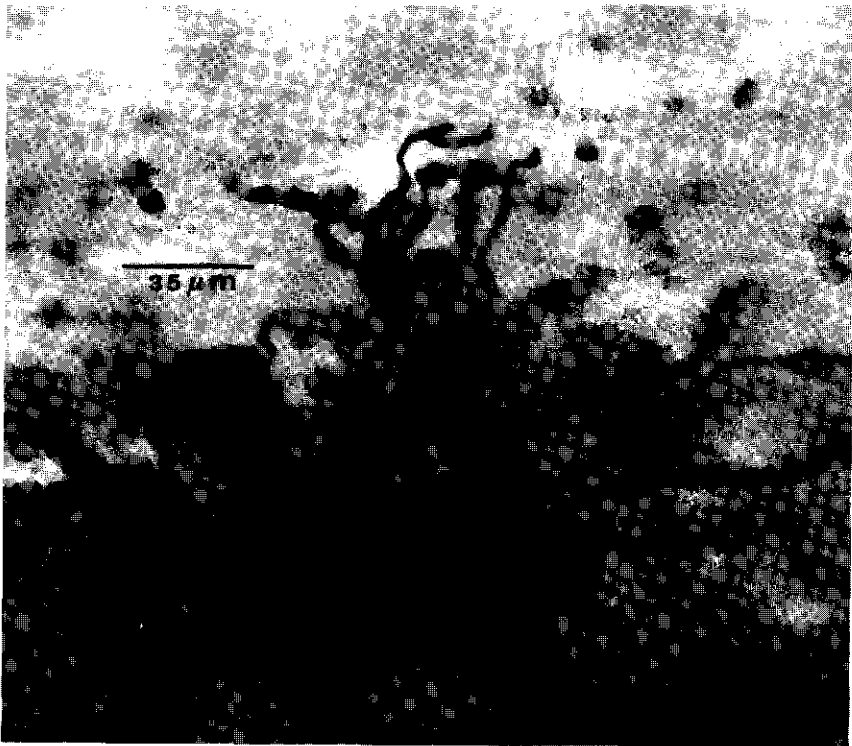

Figure 6.1 A mycelial colony of *P. punctiformis* in a root bud of *C. arvense* three weeks after inoculation

RESULTS

Resistance tests

Three weeks after inoculation, the size of *P. punctiformis* mycelial colonies varied within and between root buds. A medium sized colony is presented in Figure 6.1. The shape of the colonies often differed between the vascular tissue and the other parts of the root bud. In the vascular tissue, colonies were elongated longitudinally and they were relatively narrow. In the other parts, colonies were more circular as illustrated in Figure 6.1. In some root buds, colonies were detected at several places, indicating multiple infection. This fact was ignored in the subsequent analysis. A root bud was classified as infected or not, without a subdivision based on the number of colonies per root bud. Mycelium in root buds shorter than 0.3 cm could hardly be detected due to the undifferentiated, compact tissue. Root buds of this size were excluded from the subsequent statistical analysis. Some root buds rotted, especially the larger ones, and these were also excluded from analysis. After selection on length and rotting, 484 out of the 701 root buds (69%) were used for statistical analysis. In either experiment, significant three- and four way interactions between variables were absent, and a substantial part of the variance of root bud development and root bud infection could be explained by two-way interactions (Table 6.1). The fit of the model was better in

Table 6. 1 Interactions of hierarchical log-linear models[a] explaining variance of root bud development and root bud infection of *C. arvense* in two experiments[b].

Experiment I	Experiment II
Clone x Length	Temperature x Length
Length x Infection	Length x Infection
Clone x Infection	Clone x Infection
G = 27.7	G = 43.7
df = 34	*df* = 38
P = 0.77	P = 0.24

[a] The model was fitted to the variables clone (seven clones), length (root buds < 0.8 cm or ≥ 0.8 cm), temperature (10 or 15 °C) and infection (presence or absence of mycelium).

[b] In experiment I, the *P. punctiformis* isolate Ooy was used, in experiment II the isolate Almere.

experiment I than in experiment II as indicated by the lower G-value (and higher P-value) in experiment I. Length of root buds depended on clone in experiment I and on temperature in experiment II. This difference indicated that the two experiments differed not only with respect to the isolate, but also with respect to other (unidentified) factors. In both experiments, the fraction of root buds infected was higher in the length class ≥ 0.8 cm than in the length class < 0.8 cm. In experiment I, a fraction 0.58 of the larger root buds was infected and a fraction of 0.27 of the smaller root buds. In experiment II, a fraction of 0.44 of the larger root buds was infected and a fraction of 0.21 of the smaller root buds. In both experiments, the fraction of root buds infected depended on clone. This dependency was further analysed for between-site and within-site effects.

Except for the clones Dinteloord 0 and Dinteloord 30, the fraction of root buds infected was higher in experiment I than in experiment II (Table 6.2). The difference between the two experiments in fraction of root buds infected was significant ($G = 4.9$, $df = 1$, $P < 0.05$). In experiment I, the effect of site on the fraction of root buds infected was significant ($G = 10.1$, $df = 2$, $P < 0.01$). The fractions of root buds infected were 0.39, 0.54 and 0.33, for the sites Ooy, Almere and Dinteloord, respectively. In experiment II, these fractions were 0.23, 0.37 and 0.34, respectively, but these differences were not significant ($G = 2.4$, $df = 2$, $P > 0.05$). In both experiments, clones of the site Almere had the same ranking with respect to the fraction of root buds infected, the clone Almere 6 having the highest fraction and Almere 30 the lowest. The within-site variation of Almere was, however, non-significant in experiments I ($G = 1.4$, $df = 2$, $P > 0.05$) and II ($G = 1.1$, $df = 2$, $P > 0.05$). In both experiments, the clone Dinteloord 0 had the highest fraction of root buds infected compared to the clones Dinteloord 6 and Dinteloord 30. Within-site variation of Dinteloord was not significant in experiment I ($G = 4.8$, $df = 2$, $P > 0.05$), but significant in experiment II ($G = 15.2$, $df = 2$, $P < 0.001$).

Table 6. 2 Number of root buds free from and infected by *Puccinia punctiformis* of seven *Cirsium arvense* clones collected from three sites in The Netherlands.

		Number of root buds		
Experiment[a]	Clone[b]	Uninfected	Infected	Fraction infected
I	Ooy	23	15	0.39
	Almere 0	17	18	0.51
	Almere 6	21	31	0.60
	Almere 30	14	12	0.46
	Dinteloord 0	15	14	0.48
	Dinteloord 6	27	11	0.29
	Dinteloord 30	24	7	0.23
		141	108	0.43
II	Ooy	27	8	0.23
	Almere 0	20	12	0.38
	Almere 6	34	23	0.40
	Almere 30	22	9	0.29
	Dinteloord 0	7	10	0.59
	Dinteloord 6	13	0	0
	Dinteloord 30	33	17	0.34
		156	79	0.34

[a] The *Puccinia punctiformis* isolate Ooy was used in experiment I, the isolate Almere in experiment II.

[b] The numbers of the clones refer to the distance (m) between them at the sites Almere and Dinteloord, respectively.

DISCUSSION

This is the first study demonstrating different levels of resistance in *C. arvense* to root bud infection by *P. punctiformis*, resistance being measured as the fraction of root buds infected. Resistance as determined in the present study was quantitative (Parlevliet, 1989): the fraction of root buds infected varied between clones from zero to 0.6.
Resistance operates after parasitic contact between host and pathogen and refers to the mechanisms that interfere with and so reduce growth or development of the pathogen (Parlevliet, 1989). The measure of resistance in this study was based on a quantal response (Zadoks & Schein, 1979): development or no development of mycelium in the root bud. An additional measurement of the size of mycelium in infected root buds might have resulted in a finer measure of resistance. According to Bailiss & Wilson (1967), development of systemic infection and the typical growth disturbances of systemically infected shoots depend on the penetration of the shoot apex by the rust. Results of pot experiments (Van den Ende *et al.*, 1987) agree with the findings of Bailiss & Wilson (1967). Van den Ende *et al.* (1987) distinguished three types of systemic infection, of which two are relevant here: type A, the main shoot is systemically infected indicating that the rust has penetrated the shoot apex; type B, the main shoot is not infected and the side shoot(s) is (are) systemically infected suggesting that the rust did not reach the apex of the main shoot, but did reach the apex (apices) of the side shoot(s). It is likely that a relatively fast growing *P. punctiformis* mycelium in a root bud has a higher probability of reaching the apex of a (developing) shoot than a relatively slow growing mycelium. Testing *C. arvense* clones for the size of mycelium in the root buds may have a meaning with respect to the type of systemic infection. Determining the size of mycelium, was difficult, however, because the periphery of a mycelial colony was often insufficiently differentiated from the surrounding host tissue, especially in the vascular tissue. Perhaps, the staining and clearing method can be improved to determine differences between clones with respect to the size of mycelial colonies.
An effect of temperature on resistance could not be detected in the present experiments at 10 or 15 °C. Resistance tests at 5 and 20 °C failed so far (data not presented). After incubation at 20 °C during three weeks, root bud infection was detected, but most of the root buds had rotted, hampering an appropriate detection of mycelium. No infection was detected in root buds incubated at 5 °C during 6 weeks.
The present study demonstrated between- and within-site variation with respect to quantitative resistance of *C. arvense* to root bud infection by *P. punctiformis*. Because resistance may limit the control effect of *P. punctiformis* on *C. arvense*, the potential of

P. punctiformis to control *C. arvense* should be tested by using aggressive (Parlevliet, 1989) isolates of the rust. An extended survey and subsequent resistance tests may provide evidence for physiological races of *P. punctiformis*.

CHAPTER 7

Transport of *Puccinia punctiformis* teliospores in the soil ?

SUMMARY

To study transport of teliospores of *Puccinia punctiformis* in the soil, a method is presented to isolate teliospores from the soil. The method was tested using soil samples from a grassland site treated with *P. punctiformis* teliospores. The results are discussed with respect to the evaluation of *P. punctiformis* as a biological agent to control *Cirsium arvense*.

INTRODUCTION

The rust fungus *Puccinia punctiformis* (Str.) Röhl. is currently investigated on the potential as biological agent to control the perennial weed *Cirsium arvense* (L.) Scop. Systemic infection of *C. arvense* shoots results from placing teliospores on roots with emerging buds or on root buds (Van den Ende *et al.*, 1987; French & Lightfield, 1990). Recently, root bud infection has been demonstrated (Chapter 6). An unexplored topic is the transport of teliospores, produced on *C. arvense* shoots, to the root buds in the soil. To prove and quantify transport of teliospores in the soil, a detection method is required. Here, a detection method is presented and applied to soil samples of a grassland site in The Netherlands.

MATERIALS AND METHODS

At the site Huissen (described in Chapter 4) ten plots were randomly assigned to be sprayed with a suspension of *P. punctiformis* spores and 10 plots served as controls.

Spores were suspended using fosfolipids (NAT6008, Nattermann GmbH). The spore suspension (about 70% teliospores and 30% urediniospores by number) was sprayed onto the plots using a spray boom with nozzles of the type XR Teejet 11008 V.S. and a pressure of 2 Bar at the nozzles. The average dose per m^2 was 0.1 g spores, amounting to roughly 750 teliospores deposited per cm^2. Two weeks after spraying, the soil of the 10 sprayed plots and the 10 control plots was sampled. Cores with a diameter of 2.8 cm were taken and divided in parts of the upper 5 cm of the soil, the following 15 cm and the next following 15 cm. The three soil layers are further referred as upper, middle and lower soil layer. Ten cores per plot were taken.
The soil samples were dried in an oven (70 °C). After grinding the soil to parts smaller than 1 mm, the 10 samples collected from one soil layer of one plot were thoroughly mixed and stored. Eight subsamples of 5 ml each were taken from the mixed sample, sieved (mesh < 0.7 mm) and weighed. Each subsample was transferred to a 50 ml tube of polypropylene (Greiner). Ten ml of a bromoform-ethanol mixture, with a specific weight of 2.0, was added to the soil in the tube. The content of the tube was thoroughly mixed and an additional 5 ml of the fluid was used to wash down particles adhering to the wall. A tube was gyrated at c. 350 G during 10 minutes. The supernatant was poured into a new tube and ethanol was added until a volume of 35 ml. The tube was gyrated at 2100 G during 10 minutes. Twice, the supernatant was poured off, and ethanol was added to the precipitate until a volume of 35 ml and the tube was gyrated at 2100 G during 10 minutes. Again, the supernatant was poured off and thirty drops of glycerin were added to the precipitate in the tube. The tube was placed in an oven (40 °C) during at least one night to remove the remaining ethanol by evaporation. The content of a tube was evenly spread over four microscope slides, i.e. over an area of 70 cm^2. One of these four slides was used to count teliospores. Teliospores were counted over an area of 0.49 cm^2 , using a magnification of 150X. The numbers of teliospores of the eight subsamples per plot and soil layer were summed and converted to a number of teliospores per g oven-dry soil. The effect of spraying *P. punctiformis* teliospores on the number of teliospores isolated was analysed by ANOVA after checking the data for normality and homogeneity of variance (Sokal & Rohlf, 1981).

RESULTS AND DISCUSSION

Spraying *P. punctiformis* teliospores resulted in higher numbers of teliospores isolated from samples of the upper and middle soil layer (Table 7.1). The effect of spraying

Table 7.1. Number of teliospores determined in soil samples collected from three soil layers of control plots and plots sprayed with *Puccinia punctiformis* teliospores.

Soil layer	Number of teliospores			
	Control plots		Sprayed plots	
0 - 5 cm	26	(12)	30	(16)
5 - 20 cm	17	(10)	21	(5)
20 - 35 cm	8	(2)	8	(2)

[a] Entries are means of 9 or 10 replicated plots, standard errors of means are indicated in parentheses. Numbers of teliospores expressed per g oven-dry soil.

was, however, not significant ($P > 0.05$). The number of teliospores was relatively high in the control plots due to natural sources. The method used did not enable to determine whether the teliospores from natural sources belonged to *P. punctiformis* or to other rust species that produce teliospores with a similar morphology (e.g. *Puccinia taraxaci* (Rebent.) Plowr.). A significant difference between control plots and plots sprayed with *P. punctiformis* teliospores would have proven transport of *P. punctiformis* teliospores in soil. The lack of significance may be explained by either spraying too few teliospores compared to the numbers of teliospores produced by natural sources, or by using too low amounts of soil to isolate teliospores. Spraying a higher dose of teliospores was, however, constrained by the insufficient availability of teliospores produced under controlled conditions. Isolating teliospores from larger amounts of soil was constrained by the time investment required, i.e. 1-2 hours per soil subsample.

Evaluation of *P. punctiformis* as biological agent to control *C. arvense* using an approach of spraying teliospores onto the soil assumes transport of teliospores from soil surface to root buds. The results presented suggest that demonstation and quantification of transport of teliospores in the soil, if real at all, may be rather difficult. An approach of applying teliospores straight onto the root buds by ploughing or injection might be an alternative approach to evaluate *P. punctiformis* as biological agent to control *C. arvense*.

CHAPTER 8

General discussion

To use *P. punctiformis* for control of *C. arvense*, knowledge about the infection process resulting in systemically infected shoots is required. The available knowledge suggests various strategies to use *P. punctiformis* for biological control of *C. arvense* by increasing incidence of systemic infection.
Van den Ende *et al.* (1987) inoculated germinating seeds with teliospores, which resulted in systemically infected shoots. Spraying teliospores on germinating seeds, or seedlings, might be a strategy of biological control at the time that a site is colonized by *C. arvense*.
Menzies (1953) demonstrated growth of *P. punctiformis* mycelium from local lesions on cotyledons of *C. arvense* seedlings to the roots and subsequently into developing root buds. The results of Menzies suggest establishment of the rust in the roots during the colonization of a site by *C. arvense* seedlings, while after the phase of colonization seedlings are absent or do not survive (Amor & Harris, 1975; Van Leeuwen, 1987). Spraying urediniospores on seedlings might be another strategy for biological control at the time that a site is colonized by *C. arvense*.
Perenniality of *P. punctiformis* mycelium in the roots and subsequent invasion of newly produced root buds has been advanced in the past as a process resulting in systemically infected shoots (Olive, 1913; Bryzgalova, 1929). According to Bryzgalova, growth of the rust in the roots is very slow compared to growth of the roots, and *C. arvense* shoots may escape from systemic infection of the rust by a relatively fast growth of the roots. If so, such an escape mechanism is similar to the one proposed for the pathosystem *Lactuca siberica - Puccinia minussensis* (Wennström & Ericson, 1992). Escape from systemic infection by way of dynamic clonal growth of *C. arvense*, as found in the present studies (Chapter 4) agrees with the view of Bryzgalova (1929). Stimulating growth of mycelium in the roots by manipulating biotic or abiotic factors might be a strategy of biological control at sites occupied by established *C. arvense* populations.
Van den Ende *et al.* (1987) demonstrated that inoculation of roots, producing buds, with teliospores results in systemically infected *C. arvense* shoots. These results were confirmed by French & Lightfield (1990) who inoculated root buds with teliospores. The results of Van den Ende *et al.* (1987) and French & Lightfield (1990) suggested root bud infection, a suggestion proved in Chapter 6. Inoculation of root buds with teliospores might be a strategy of biological control at sites occupied by established *C. arvense* populations. This strategy is at the centre of the present thesis.

The present studies provide evidence that root buds may be infected at the end of the summer or in the autumn and provide clues to increase root bud infection in those periods. Infection of root buds in those periods may be required to achieve a relatively

high incidence of systemic infection early in the growing season as suggested by results of the matrix population model (Chapter 4).

Deposition of *P. punctiformis* teliospores is temporally concentrated in the autumn (Chapter 2). Progress curves of teliospore deposition indicated an onset of teliospore deposition in July, a mid deposition time at the beginning of October and a cessation near the end of October. The spatial distribution of teliospores was non-uniform, and therefore the probability of contact between teliospores and root buds may vary within a site. Variation in the probablity of contact between root buds and teliospores is a presumable explanation of the spatial variation determined at the study sites (Chapter 2, 3). To prevent spatial escape of root buds from infection, control of *C. arvense* by *P. punctiformis* may require a spatially homogeneous distribution of teliospores in autumn.

After deposition on the soil, *P. punctiformis* teliospores have to be transported into the soil to result in root bud infection. An experiment at the end of the summer could not prove transport of *P. punctiformis* teliospores into the soil (Chapter 7). Root buds might escape from infection by an inadequate transport of teliospores in the soil. Control of *C. arvense* by *P. punctiformis* may, therefore, depend on transport of teliospores in the soil.

Teliospores germinate in the range of 5-20 °C, suggesting root bud infection in this range of temperature (Chapter 5). Root bud infection was demonstrated at 10, 15 and 20 °C but not at 5 °C(Chapter 6). A soil temperature in the range of 10-15 °C was common at the research sites at the end of the summer and in the autumn (data not presented). The temperature was measured at 10 and 30 cm depth in the soil. Dependent on site and year, root buds were present in the upper 20 or 40 cm of the soil during the whole year (data not presented). With respect to temperature, root buds may be infected at the end of the summer and in the autumn. An interaction between temperature, clones and root bud infection, i.e. an indication for avoidance, could not be detected in the infection experiments (Chapter 6). More extensive tests are, however, required to confirm the results of these experiments.

At 10 and 15 °C, resistance may limit root bud infection and subsequent development of systemic infection (Chapter 6). Site-specific, quantitative resistance of *C. arvense* to root bud infection was determined using the foreign isolate Ooy to test clones of the sites Ooy, Almere and Dinteloord. Using the isolate Almere, in another experiment, within-site variation of resistance was demonstrated for the site Dinteloord and not for the site Almere. The numbers of clones and isolates tested were too few to explain epidemiological processes at the research sites. Within-site variation of resistance at the site Dinteloord and not at the site Almere would, however, agree with the relatively strong hierarchy of the pathogen population at the site Dinteloord and the relatively weak hierarchy at the site Almere (Chapter 3). The demonstration of site-specific resistance suggests that the use of one *P. punctiformis* isolate may be insufficient to control *C. arvense* at a large geographic scale. The quantitative character of resistance

suggests polygenic inheritance of resistance. If so, selection and breeding of aggressive *P. punctiformis* strains for biological control of *C. arvense* might be complicated.

Field experiments of spraying *P. punctiformis* teliospores, isolate Ooy, in the autumn of 1990 and 1991 did not result in a higher incidence of systemic infection in the following spring at the sites Almere, Dinteloord and Yerseke (Frantzen & Scheepens, 1993). Teliospores were evenly distributed over the experimental sites to prevent spatial escape of root buds from infection. The number of teliospores sprayed was equal to or exceeded the numbers of teliospores produced by natural sources. A too low or spatially unequal distribution number of teliospores seems unlikely as an explanation for the failure of these field experiments. The results of the infection experiments (Chapter 6) suggest resistance as an explanation, the agressiveness of the isolate used might have been insufficient. This hypothesis was tested for clones sampled at the sites Almere and Dinteloord. The results of these tests indicated that resistance may at best partially explain the lack of success of the field experiments. Another constraint to root bud infection might have been a lack of transport of teliospores in the soil after spraying teliospores. As stated in Chapter 7, ploughing or injection of teliospores in the soil might be an alternative to spraying teliospores and subsequent transport into the soil.

The results of the present thesis may be abstracted in more general terms that describe factors reducing the impact of pathogenic fungi on host populations, to facilitate comparison with other pathosystems (Table 8.1). Escape is a common point of interest for biological weed control using fungi (Shrum, 1982). Shrum used the term escape in a broad sense, i.e. including avoidance. A distinction between escape and avoidance may, however, have meaning. In the case of escape (*sensu stricto*), a method of biological control has to take into account heterogeneous environmental factors. In the case of avoidance, heterogeneity of the weed population has to be taken into account. The solution to the problem of escape (*sensu lato*) is application of the bioherbicide at the right time(s) and location(s), a common practice to increase the impact of a fungus on the weed population (TeBeest *et al.*, 1992). Another solution to the problem of avoidance might be selection of or breeding for fungal strains with high infectivity.

Table 8.1 Factors that may reduce the impact of *Puccinia punctiformis* on populations of *Cirsium arvense*. For explanation see text.

Escape	demonstrated	Chapter 4
Avoidance	not demonstrated	Chapter 6
Resistance	demonstrated	Chapter 6
Tolerance	not demonstrated	-
Compensation	suggested	Chapter 4

Resistance is a common point of interest for biological weed control (Shrum, 1982), but was, probably due to a fortunate selection of fungal strains, no major constraint for the development of *Colletotrichum gloeosporioides* (Penz.) Penz. *et* Sacc. f.sp. *aeschynomene* (trade mark Collego) against *Aeschynomene virginica* (L.) B.S.P. and of *Phytophthora palmivora* (Butl.) Butl. (trade mark DeVine) against *Morrenia odorata* Lindl. (Charudattan, 1988). No reports addressed to resistance are available of other weeds controlled by bioherbicides. In general, selection of or breeding for pathogenic fungal strains has been proposed as a strategy to deal with resistance of weeds (Leonard, 1982).

Tolerance has not yet been considered with respect to biological weed control using fungi. Development and use of a method to test *C. arvense* on tolerance may demonstrate whether some phenotypes suffer less from systemic infection by *P. punctiformis*, i.e. are more difficult to control.

So far, no study is known to address specifically the consequences of inter-plant and between-plant compensation for the use of fungi as bioherbicides. As discussed for the pathosystem *C. arvense* - *P. punctiformis* (Chapter 4), compensation might be a point of interest for biological weed control.

The present thesis reflects an approach of dovetailing plant population biology and phytopathology. Phytopathology is concerned with the prevention of crop losses due to pathogens in agricultural plant populations, and plant population biology with the understanding of the causes of changes in numerical abundance and gene frequency in wild plant populations (Antonovics & Alexander, 1989). In line with this separation of the two research fields, pathosystems were divided by Robinson (1976) in artificial (crops) and natural (wild plants). Pathogens with weeds as hosts are at the borderline of natural and artificial. Alexander (1989) compared agricultural and natural plant populations in general. Adapting her comparison, a weed population may be considered as agricultural with respect to the relatively uniform physical environment and the strong human influence on the plant population characteristics. In spatial variability with respect to the developmental stage and in variability with respect to plant spacing they resemble natural plant populations. The spatial distribution of the host population and the spatial differences in other biotic factors and abiotic factors may have an effect on the intensity of pathogenic infection (Burdon *et al.*, 1989; Alexander, 1989). Because of the differences between weed populations and agricultural plant and wild plant populations, and the effects of these differences on pathogenic infection, a classification of weeds and their pathogens as natural or as artificial pathosystem seems to be invalid. The interest in the use of pathogens for biological weed control (e.g. TeBeest, 1991) supports the introduction of a new system term for weeds and their pathogens. Here, the term weed pathosystem is proposed, and, adapted from Robinson's definition of pathosystem (1976), defined as: any sub-system of the ecosystem which involves parasitism causing diseases of weeds.

Knowledge about weed pathosystems in general and about the use of pathogens as bioherbicides more specifically is limited. The weed pathosystem *C. arvense* - *P. punctiformis* was investigated in a rather broad way by considering factors ranging from escape to compensation (table 8.1). The present thesis could not demonstrate relevance of the factors avoidance and tolerance in weed pathosystems. The factor compensation is suggested as relevant in weed pathosystems, the importance of the factor escape is confirmed and the importance of resistance is demonstrated. To evaluate the potential of *P. punctiformis* as bioherbicide against *C. arvense*, further studies are required. A first point of interest for the subsequent studies would be escape, more specifically by lack of transport of teliospores in the soil. A second point of interest would be selection of aggressive *P. punctiformis* strains. When the incidence of systemic infection can be managed, the factor compensation requires attention. The subsequent studies may find their base in the framework that emerges in this thesis.

Summary

The biology and epidemiology of the rust fungus *Puccinia punctiformis* (Str.) Röhl. was investigated to evaluate the potential of this rust as a biological agent against the clonal plant species *Cirsium arvense* (L.) Scop., which is considered world-wide as a weed. Incidences of systemic infections and local lesion infection cycle were quantified (Chapter 2). The spatial variation of incidences within *C. arvense* populations were expressed in the Gini coefficient, a measure adopted from plant population biology. Incidence of systemic infection was low and spatially heterogeneous compared to the wide-spread occurrence of local lesion infection within *C. arvense* populations. Systemic infections are far more damaging to *C. arvense* shoots than local lesions. The present studies were, therefore, focussed on the increase of systemic infection incidence using teliospores as inoculum.
Deposition of teliospores, produced on systemically infected shoots and shoots with local lesions, occurs mainly in the autumn (Chapter 2). The temporal distribution of teliospore deposition could be described by progress curves with an onset of deposition in July, a mid deposition time (50% of the teliospores deposited) at the beginning of October, and a cessation near the end of October. The average of the maximum deposition was estimated at about 1000 teliospores cm^{-2}, but varied over the site.
Deposition of teliospores around shoots with local lesions decreased with distance from these shoots (Chapter 2). The half distance α (range from the shoot in which 50% of the teliospores is deposited) was estimated to be 6.1 cm. This relatively short half distance suggests that teliospores are mostly deposited in the immediate vicinity of shoots with local lesions. The study of teliospore deposition around isolated *C. arvense* shoots was extended to a spatial analysis of teliospore deposition in two plots of 4 m by 4 m. In both plots, teliospores deposition was spatially heterogeneous (Gini coefficients of 0.50 and 0.57, respectively). In one plot, deposition was spatially non-random (Moran's I about 0.40) and the semivariogram suggested a pattern of focus formation. The time required for spread of local lesion infection seemed to determine the spatial distribution of teliospores. In another plot, no deviation from a random spatial distribution of teliospores could be detected. In both plots, the spatial distribution of *C. arvense* root buds, the ports of entry for the rust, was heterogeneous (Gini coefficients about 0.4). A specific spatial pattern of root bud distribution could not be detected. A comparison of the spatial distributions of teliospore deposition and of root buds suggested that the probability of contact between root buds and teliospores varies over a site, so that root buds may escape from infection.
Spatial analysis of teliospore deposition was restricted to one of the study sites. A comparison of *P. punctiformis* populations indicated that incidence of *P. punctiformis* in *C. arvense* populations is site specific (Chapter 3) and suggests that a spatial analysis of teliospore deposition at more sites is required to confirm the results of the spatial analysis presented. The comparison of *P. punctiformis* populations, which consist of

individuals in different stages of the infection cycle, based on a concept of variability profiles, adopted from systematic zoology. The study demonstrated that the smaller *P. punctiformis* populations, the more individuals were concentrated at a few places within a site. So, a strong spatial inequality of biotic or abiotic conditions influenced development of *P. punctiformis* populations at those sites.

A biotic factor that causes within-site variation of systemic infection is the type of clonal growth of *C. arvense* (Chapter 4). A population dynamics study of *C. arvense* shoot populations demonstrated that shoots produced by 'static' clonal growth have a higher probability to be systemically infected than shoots produced by 'dynamic' clonal growth. Static clonal growth was characterized by occurrence of *C. arvense* shoots in a cell of 25 cm by 25 cm in two subsequent years, whereas dynamic clonal growth was characterized by occurrence of shoots in a cell that was not occupied by *C. arvense* shoots in the preceding year. Dynamic clonal growth may be a mechanism of escape from systemic infection.

Resistance of *C. arvense* to root bud infection, and subsequent development of systemic infection, was investigated as a biotic factor that may explain spatial variation of systemic infection within and between sites. Germination experiments were conducted to design resistance tests appropriately (Chapter 5). Germination progress curves were estimated for 5, 10, 15 and 20 °C . The mid germination time (the time at which 50% of the germinable teliospores are germinated) was shortest at 15 °C (6 days) and longest at 5 °C (about 20 days). The rate of germination at the mid germination time was highest at 10 and 15 °C. The results of the germination experiments suggest a higher rate of root bud infection at 10 and 15 °C than at 5 and 20 °C. The subsequent infection experiments demonstrated root bud infection at 10, 15 and 20 °C (Chapter 6). No infection could be detected in root buds incubated at 5 °C during six weeks. The infection experiments conducted at 10 and 15 °C demonstrated quantitative resistance of *C. arvense* to root bud infection. Dependent on clone, the fraction of root buds infected varied between 0.0 and 0.6. Within- and between-site effects on quantitative resistance were determined.

A method is presented to isolate teliospores from soil (Chapter 7). Transport of *P. punctiformis* teliospores in the soil could not be demonstrated after spraying teliospores at the end of the summer. The results suggest that root buds may escape from infection by an inadequate transport of teliospores in the soil.

The results of the present studies are summarized and discussed using a scheme of major factors that may limit the impact of a pathogen on a host population, i.e. escape, avoidance, resistance, tolerance and compensation (Chapter 8). The relevance of escape and resistance were demonstrated and the relevance of compensation is suggested. Subsequent studies are proposed on base of the framework that emerges from the present studies. The term weed pathosystem is defined to distinguish the specific characters of weed populations, and their effects on infection, from those of crop and wild plant populations.

Samenvatting

De biologie en epidemiologie van de roestschimmel *Puccinia punctiformis* (Str.) Röhl. is onderzocht ten behoeve van de biologische bestrijding van *Cirsium arvense* (L.) Scop., de akkerdistel. De infectiecyclus van *P. punctiformis* omvat systemische infectie van distelscheuten en lokale lesies op stengels en bladeren. Systemische infectie heeft, in tegenstelling tot lokale lesies, een sterk negatief effect op de geïnfecteerde distelscheut. De fractie systemisch geïnfecteerde scheuten in distelpopulaties is relatief laag (bevestigd door resultaten van dit proefschrift). Gelet op het beperkte vóórkomen en het sterk negatieve effect op distelscheuten van systemische infectie, lijkt het verhogen van de fractie systemisch geïnfecteerde scheuten een geschikte strategie voor de biologische bestrijding van de akkerdistel met de roestschimmel. Om een basis te bieden voor een dergelijke strategie is de verspreiding van *P. punctiformis* teliosporen onderzocht, de ruimtelijke verdeling van akkerdistel in relatie tot de kans op systemische infectie én het infectieproces leidend tot systemische infectie.
Het inoculeren van wortels van de akkerdistel met teliosporen leidt, via knopinfectie, tot systemisch geïnfecteerde scheuten. Teliosporen worden zowel op systemisch geïnfecteerde scheuten als op stengels en bladeren met lokale lesies gevormd. Uit het veldonderzoek bleek dat teliosporen vooral in het najaar op de bodem terechtkomen (hoofdstuk 2). De verdeling van de teliosporen-depositie in de tijd was weer te geven met een log-logistische curve. Depositie van teliosporen begon in juli, de mid-depositie tijd (50% van de teliosporen is op de grond gevallen) viel aan het begin van oktober en de depositie nam af naar het eind van oktober. Gerekend over de gehele periode vielen gemiddeld circa 1000 teliosporen per cm^2 op de grond.
De depositie van teliosporen rond scheuten met lokale lesies nam af met toenemende afstand tot deze scheuten (hoofdstuk 2). Een mid-depositie afstand (50 % van de teliosporen valt binnen deze afstand op de grond) van 6.1 cm werd vastgesteld. Deze relatief korte afstand suggereert dat teliosporen vooral in de onmiddelijke nabijheid van scheuten met lokale lesies op de grond vallen. Het bepalen van de depositie van teliosporen rond geïsoleerde, geïnfecteerde, scheuten werd aangevuld met een ruimtelijke analyse van teliosporen-depositie in twee proefvlakken van vier bij vier meter elk. In beide vlakken vertoonde de depositie van teliosporen ruimtelijke variatie (waarden voor de Gini coefficient van respectievelijk 0.50 en 0.57). In één van de vlakken was de ruimtelijke variatie niet 'random' verdeeld (Moran's I van 0.40) maar vertoonde een patroon van 'haard'-vorming. De 'haard'-vorming was te beschrijven met een semivariogram. In het betreffende vlak zou de beperkte tijd, beschikbaar voor de polycyclische verspreiding van lokale lesies, het ruimtelijk patroon in teliosporen-depositie veroorzaakt kunnen hebben. In het andere proefvlak was geen afwijking van een 'random' verdeelde teliosporen-depositie te ontdekken. In beide proefvlakken is ook het ruimtelijke patroon van wortelknoppen van de akkerdistel bestudeerd, de infectiepoorten voor de roestschimmel. Het aantal wortelknoppen varieerde binnen een proefvlak (waarden voor de Gini coefficient van circa 0.40) maar in geen van de beide

vlakken was een duidelijk ruimtelijk patroon te ontdekken. Uit een vergelijking van de ruimtelijke verdeling van teliosporen en van wortelknoppen was af te leiden dat de trefkans tussen wortelknoppen en teliosporen ongelijk verdeeld kan zijn binnen de ruimte van een grasland. Wortelknoppen kunnen aan systemische infectie ontsnappen door het ontbreken van contact met teliosporen ('ontsnapping').
De ruimtelijke analyse van teliosporen-depositie was beperkt tot één van de graslanden die voor het onderzoek gebruikt zijn. Een vergelijking van *P. punctiformis* populaties in de verschillende graslanden liet zien dat de mate van vóórkomen van *P. punctiformis* in distelpopulaties sterk verschilt tussen graslanden (hoofdstuk 3). De vergelijking van *P. punctiformis* populaties was gebaseerd op het concept van 'variatie profielen' ('variability profiles'), een concept dat binnen de zoölogische taxonomie wordt toegepast (hoofdstuk 3). Het vergelijken van *P. punctiformis* populaties liet zien dat naarmate de populatie kleiner was de individuen van een populatie meer geconcentreerd waren op enkele plekken binnen een grasland. Een kleine, op enkele plekken in een grasland geconcentreerde *P. punctiformis* populatie kan veroorzaakt zijn door biotische en/of abiotische factoren die ruimtelijk sterk varieren.
Het type klonale groei van distelplanten bleek een biotische factor te zijn die ruimtelijke variatie in systemische infectie binnen een grasland kan veroorzaken (hoofdstuk 4). De populatiedynamische studie liet zien dat distelscheuten geproduceerd via 'statische' klonale groei een grotere kans hebben om systemisch geïnfecteerd te raken dan scheuten geproduceerd via 'dynamische' klonale groei. Statische klonale groei werd gekenmerkt door het aanwezig zijn van distelscheuten in een vlakje van 25 bij 25 cm dat ook in het voorgaande jaar was bezet met distelscheuten. Dynamische klonale groei werd gekenmerkt door de aanwezigheid van scheuten in een vlakje dat in het voorgaande jaar niet begroeid was met distelscheuten. Dynamische klonale groei lijkt te fungeren als mechanisme om aan systemische infectie te ontsnappen.
Een biotische factor die zowel variatie in systemische infectie binnen een grasland als variatie tussen graslanden kan veroorzaken is resistentie, de afweer van planten tegen ziekte. Om de factor resistentie adequaat te kunnen onderzoeken, zijn eerst kiemingsexperimenten met teliosporen uitgevoerd (hoofdstuk 5). Log-logistische curven van de kieming van teliosporen in de tijd werden uitgezet voor 5, 10, 15 en 20 °C. De mid-kiemingstijd (de helft van de kiemkrachtige teliosporen is gekiemd) was het kortste bij 15 °C (6 dagen) en het langste bij 5 °C (20 dagen). De toename van de fractie gekiemde teliosporen op de mid-kiemingstijd was het sterkst bij 10 en 15 °C. De resultaten van de kiemingsexperimenten suggereren dat binnen een zekere tijdsduur meer knopinfecties kunnen optreden bij 10 en 15 °C dan bij 5 en 20 °C. In de infectie-experimenten werd knopinfectie aangetoond bij 10, 15 en 20 °C maar niet bij 5 °C (hoofdstuk 6). In de infectie-experimenten die bij 10 en 15 °C werden uitgevoerd, is kwantitatieve resistentie van de akkerdistel tegen knopinfectie gevonden. Al naar gelang de distelkloon werd een fractie van 0.0 tot 0.6 van de wortelknoppen door de roestschimmel geïnfecteerd. De mate van resistentie varieerde tussen klonen verzameld in hetzelfde grasland én tussen klonen verzameld in verschillende graslanden.

In hoofdstuk 7 werd een aanzet gegeven voor onderzoek naar het transport van *P. punctiformis* teliosporen in de bodem met een methode om teliosporen uit de bodem te isoleren. In een eenmalig uitgevoerd experiment kon transport van teliosporen in de bodem echter niet worden aangetoond. Het blijft onduidelijk in hoeverre transport van teliosporen in de bodem een belemmering is voor knopinfectie.
In hoofdstuk 8 worden de resultaten van het onderzoek in breder verband besproken. De term onkruid-pathosysteem wordt geïntroduceerd om de specifieke kenmerken van onkruidpopulaties en de daarmee samenhangende ontwikkeling van pathogenen te onderscheiden van gewaspopulaties en populaties van wilde planten met hun respectievelijke pathogenen. Zowel in gewas-pathosystemen als wilde-pathosystemen is resistentie een bekend verschijnsel. Resistentie is niet gevonden in de onkruid-pathosystemen die tot nu toe bestudeerd zijn bij het ontwikkelen van pathogenen als onkruidbestrijdingsmiddel. Kwantitatieve resistentie blijkt een rol te spelen in het onkruid-pathosysteem *C. arvense* - *P. punctiformis*. De factor 'ontsnapping' in onkruid-pathosystemen is reeds eerder als drempel voor het ontwikkelen van biologische onkruidbestrijdingsmiddelen onderkend. Uit vervolgstudies, gebaseerd op resultaten van dit proefschrift, moge blijken of de drempels van resistentie en 'ontsnapping' te overwinnen zijn en biologische bestrijding van de akkerdistel met *P. Punctiformis* mogelijk is.

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List of publications

Frantzen, J. An epidemiological study of *Puccinia punctiformis* as a stepping stone to biological control of *Cirsium arvense*. Submitted to New Phytologist.

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Curriculum vitae

Jos Frantzen, geboren 19 februari 1960, werd bij de gemeente Maastricht ingeschreven met de voornamen Pieter Anna Maria Jozef maar bij de Rooms-Katholieke kerk in de volgorde Pieter Jozef Anna Maria. Het gymnasium 'Rolduc' te Kerkrade werd afgerond in 1978 en de studie biologie aan de KU Nijmegen in 1984. Zwaartepunten van de studie biologie waren geobotanie, bodemkunde en rechten. Een driemaands-stage in 1983 bij de Zwitserse vestiging van het Commonwealth Institute of Biological Control (UK) in Delémont was de eerste kennismaking met biologische bestrijding van onkruiden. In 1984 en 1985 werd de vervangende dienstplicht vervuld bij de wetenschapswinkel van de Vrije Universiteit. In 1986 werd een extra bijvak fytopathologie gevolgd aan de Landbouwuniversiteit Wageningen. Binnen dit bijvak werd stage gelopen bij het CABO-DLO met als onderwerp de biologische bestrijding van knolcyperus met de roestschimmel *Puccinia canaliculata*. Eind 1987 volgde een aanstelling bij het CABO-DLO voor de uitvoering van het project 'Biologische bestrijding van akkerdistel met de roestschimmel *Puccinia punctiformis*'. In 1993 wordt met een post-doctorale opleiding tot epidemioloog A aan de Vrije Universiteit begonnen.