Infection process and resistance in the weed pathosystem *Senecio vulgaris* – *Puccinia lagenophorae* and implications for biological control

Gabriela S. Wyss and Heinz Müller-Schärer

Abstract: The infection process and the level and type of resistance in the pathosystem of a native weed and a naturalized rust fungus, *Senecio vulgaris* L. – *Puccinia lagenophorae* Cooke, were examined. Four inbred plant lines from Switzerland (2), The Netherlands, and the United Kingdom, each at two stages of devolpment, were exposed to corresponding rust lines under controlled conditions. Fluorescence and light microscopy were used to assess the infection process and to quantify genotype effects. Component analysis was used to partition disease development and define the infection process. Germinating aeciospores of *P. lagenophorae* showed all the characteristics of the monokaryotic parasitic stage of rust infection with direct penetration and monokaryotic haustoria formation. Haustoria formed between 3 and 6 days after inoculation at low frequency. The highest level of resistance, for which differences between plant lines were detected, occurred at penetration peg formation. All the studied host–pathogen interactions were compatible. The Dutch plant line was most susceptible to all rust lines, and the Dutch rust line was the most aggressive. Susceptibility of leaves increased with the leaf developmental stage. There was a continuous range of variation in susceptibility without differential genetic interactions, thus indicating race-nonspecific quantitative resistance. The use of the more aggressive Dutch rust line may increase the level of disease, thus stimulating epidemics for biological control. Over the long term, less susceptible genotypes of the weed may be selected, although differences in susceptibility among plant lines were relatively small.

Key words: rust fungus, infection structures, direct penetration, component analysis, race-nonspecific quantitative resistance.

Résumé : Les auteurs ont examiné le processus d'infection ainsi que le niveau et le type de résistance dans le pathosystème d'une mauvaise herbe et d'une rouille fongique naturalisée, *Senecio vulgaris* L. – *Puccinia lagenophorae* Cooke. Les auteurs ont exposé quatre lignées de plantes autofécondées provenant de Suisse (2), de Hollande et d'Angleterre, chacune à deux stades de développement, aux lignées de rouille correspondantes, sous des conditions contrôlées. Ils ont utilisé la microscopie photonique et en fluorescence pour évaluer le processus d'infection et pour quantifier les effets des génotypes. Ils ont aussi utilisé l'analyse par composantes pour diviser le développement de la maladie et définir les processus d'infection. Les aeciospores en germination de *P. lagenophorae* montrent toutes les caractéristiques du stade parasitaire monocaryotique de l'infection par la rouille avec la pénétration directe et la formation d'haustéries monocaryotes. Les haustéries se forment entre 3 et 6 jours après l'inoculation à faible fréquence. Le degré le plus élevé de résistance, pour lequel on observe des différences entre les lignées de plantes, se manifeste au moment de la formation de la cheville d'infection. Toutes les interactions hôte–pathogène étudiées sont compatibles. La lignée de plante hollandaise s'est avérée la plus susceptible à toutes les lignées de rouille et la lignée hollandaise de rouille est la plus aggressive. La susceptibilité des feuilles augmente avec le stade de développement foliaire. Il y a une gamme continue de variation dans la susceptibilité sans interactions génétiques différentielles, ce qui indique une résistance quantitative non-spécifique à la race. L'utilisation de la lignée de rouille hollandaise plus aggressive peut augmenter le degré de maladie, stimulant ainsi les épidémies en vue de la lutte biologique. À long terme, on pourrait sélectionner des génotypes moins susceptibles de la plante hôte, bien que les différences de susceptibilité entre les lignées de plantes soient relativement faibles.

Mots clés : rouille fongique, structures d'infection, pénétration directe, analyse par composantes, résistance quantitative non-spécifique à la race.

[Traduit par la Rédaction]

Received August 9, 1998.

G.S. Wyss.[1] Swiss Federal Research Station of Fruit-Growing, Viticulture and Horticulture, CH-8820 Wädenswil, Switzerland.
H. Müller-Schärer. Département de Biologie/Ecologie, Université de Fribourg, Pérolles, CH-1700 Fribourg, Switzerland.

[1]Author to whom all correspondence should be addressed. Present address: 1453 Fifield Hall, P.O. Box 110680, Plant Pathology Department, University of Florida, Gainesville, FL 32611-0680, U.S.A. e-mail: wyss@gnv.ifas.ufl.edu

Introduction

A pathosystem is an ecosystem which involves parasitism and may be natural (wild pathosystem) or artificial (crop pathosystem) (Robinson 1976). The interest in studying plant–pathogen interactions in natural plant communities has increased during the last two decades, mainly because of the search for disease resistance genes to gain a better understanding of disease epidemics (Dinoor and Eshed 1984). Furthermore, such studies are important where natural enemies are deliberately used in a biological control program to reduce and stabilize the density of a target weed population below an economic threshold (Charudattan 1988). In crops, weed pathosystems may have characteristics of both natural and crop pathosystems as some of them originated in natural plant populations and have adapted to a crop habitat.

One form of biological control is the system management approach (Müller-Schärer and Frantzen 1996). The weed pathosystem is manipulated to shift the balance between the host and an indigenous pathogen population in favour of the pathogen. Weed control is achieved by stimulating the buildup of a disease epidemic on the target weed population, thus reducing the competitiveness of the weed. This approach might be suitable where immediate and complete control of the weed is not required, where the natural enemy cannot be mass-produced, and where the introduction of an exotic natural enemy is excluded. Therefore, this approach complements the bioherbicide (Charudattan 1988) and the classical approaches (Watson 1991) of weed biocontrol.

Resistance in host plant populations may delay or slow epidemics. This could be a limiting factor for successful application of biological control with the system management approach and the other methods. Resistance mechanisms operate after parasitic contact between the host and the pathogen, to reduce the development and (or) growth of the pathogen (Parlevliet 1989). For the purpose of this study, resistance is expressed as differences among plant lines in susceptibility to the pathogen. In case of qualitative resistance, differences between plant lines can be separated into discrete categories that do not overlap and are governed by one or a few effective resistance genes (Ennos 1992). Quantitative resistance shows a continuous range of variation from no resistance (extremely susceptible) to good levels of resistance (incomplete resistance). Environmental conditions may influence quantitative resistance relatively strongly (Parlevliet 1989). Specific interactions among plant and pathogen lines may be determined for both qualitative and quantitative resistance.

Differences among host genotypes or among pathogen isolates can be found with respect to various phases of the infection process such as spore germination, penetration, colonization, and sporulation (Zadoks and Schein 1979). In this context, component analysis is a quantitative approach aimed at partitioning disease defence into its components and to define the stage of the infection process at which the defence mechanism of the plant becomes effective and fungal growth is inhibited (Zadoks and Schein 1979). Jacobs et al. (1996), for instance, used components of resistance to *P. recondita* f.sp. *tritici* to define differences in various host genotypes and plant developmental stages of bread wheat, *Triticum aestivum* L., its resistant parent line, and susceptible recurrent parent line *Triticum monococcum* L. Component analysis was reported first for cereal–rust relationships (Zadoks 1972) and only recently for herbaceous plants (Hatcher et al. 1995).

We have chosen the weed pathosystem *Senecio vulgaris* – *Puccinia lagenophorae* as a research model (Müller-Schärer and Frantzen 1996). *Senecio vulgaris* is a self-fertile and strongly self-pollinating annual (Kadereit 1984) and considered as a weed in Switzerland (Müller-Schärer and Wyss 1994) and many other countries (Holm et al. 1997). This is mainly due to resistance to s-triazine herbicides (Holt and LeBaron 1990) and the use of herbicides with limited range of activity (G. Wyss, personal observation). Besides, it shows the characteristics of a successful weed invader (Kadereit 1984). Plants of *S. vulgaris* may compete strongly with crops for resources (Müller-Schärer and Rieger 1998; Paul and Ayres 1987).

The autoecious rust fungus *P. lagenophorae*, widely distributed in Australia, was first detected in Europe on *S. vulgaris* in the early 1960s (Viennot-Bourgin 1964). Since then it has spread rapidly from France to the United Kingdom (Wilson et al. 1965), Ireland (Kavanagh 1964), and to whole of central Europe (Scholler 1993 and 1997). It is now common throughout Europe. The life cycle of *P. lagenophorae* on *S. vulgaris* lacks the stages of uredinia and pycnia in Europe even though the latter might be expected because of the presence of basidiospores (N. Paul, University of Lancaster and J. Frantzen, University of Fribourg, personal communication). The rust colonizes leaves, stems, and capituli by way of typical orange colored aeciospores and causes severe malformations and distortions.

The physiological consequences of the rust infection on *S. vulgaris* were reviewed by Paul et al. (1993). The potential of *P. lagenophorae* infection to reduce the competitiveness of *S. vulgaris* plants and, thus, maintain crop yield has been demonstrated by Paul and Ayres (1987) for lettuce (*Lactuca sativa* L.) and by Müller-Schärer and Rieger (1998) for celeriac (root celery, *Apium graveolens* L.). The present knowledge of biolgical control of *S. vulgaris* has been compiled recently by Frantzen and Hatcher (1997). To develop the system management approach further on *S. vulgaris* using the rust fungus *P. lagenophorae*, the following objectives were addressed in this study: description of the infection process to divide it into successive stages and to localize resistance mechanisms (experiment 1); quantification of the effects of plant and rust lines from a wide geographic range using component analysis (experiment 2); and determination of host–pathogen interactions to identify the type of resistance mechanisms involved (experiment 3). The implications of the results for biological control of *S. vulgaris* are discussed.

Materials and methods

Plant and fungal material

Full sibship plant lines of *S. vulgaris* (when outbreeding did not occur) were used from Unterehrendingen, Switzerland (referred to as pCH I), from Charrat, Switzerland (pCH II), from Leiden, the Netherlands (pNL II) and from Lancaster, U.K. (pUK). The plant lines were cultured under similar conditions in the climate room as

described below (see infection process) for one to four generations to eliminate potential maternal effects because of differences in environmental conditions among the sites of origin.

Pure rust lines were obtained from single-aeciospore cultures maintained on host plants of the corresponding origin, separated by transparent plastic boxes. They were cultured under controlled conditions as described below (see infection process). The Swiss rust lines rELS and rCH I, the Dutch line rNL II, and the British line rUK were all collected from the corresponding plant population from which the plant lines were selected, except the Dutch rust line, which had its origin in an orchard at Lienden, the Netherlands. Aeciospores were applied freshly or after storage at –18°C in Eppendorf vials.

Infection process (experiment 1)

The plant line pCH II and the rust line rELS were used to study the infection process. Seeds were germinated in plastic trays containing nutrient-amended peat (Floragard, TKS 2) and transplanted individually into 9 cm diameter pots when they were at the 4-leaf stage. Plants were grown under controlled conditions at 23 ± 1°C in the day and 17 ± 1°C at night, at relative humidity (RH) of 40–60% and a photoperiod of 16 h light (260 $\mu mol \cdot m^{-2} \cdot s^{-1}$) : 8 h dark. Plants at the six-leaf stage were selected for use in experiment 1.

For studying infection structures formed within 2 days after inoculation, discs of 3.14 cm^2 were punched out from the third and fourth true leaf of a plant using a stainless steel cork-borer. The discs were inoculated with aeciospores using a camel-hair brush. The leaf discs were placed in a tray on wet blotting paper and covered with transparent plastic. They were kept at 100% RH in the dark at 18 ± 1°C and stained (see below) at 4-h intervals until 48 h after inoculation.

For studying infection structures formed 2 days after inoculation, plants were inoculated with a suspension of 0.5 mg aeciospores in 1 mL distilled water using a DeVilbiss hand atomizer. The plants were placed in a tray and covered by a transparent plastic bag during the night at 17 ± 1°C. After removing the plastic bag, plants were grown under the conditions mentioned above. Discs of 3.14 cm^2 were punched out from the third and fourth true leaf of a plant, which is the second true leaf pair, at 3, 6, and 9 days after inoculation and were stained immediately.

Some leaf discs were stained using a modified method of Bruzzese and Hasan (1983). To allow for the thin leaf structure of *S. vulgaris* plants, tissue was placed for 24 h (instead of 48 h) in the decolorizing–staining solution and cleared in the concentrated chloral hydrate solution over 20 h (instead of 24 h).

Other leaf discs were stained following Rohringer et al. (1977) as modified by Kuck et al. (1981) for better dye effects. The optical brightener calcofluor was replaced by 0.2% (w/v) diethanol (Uvitex 2B, Ciba Geigy, Basel, Switzerland).

All leaf samples were mounted in polyvinyl alcohol (Omar et al. 1978). Observations were made with a light microscope (Leica, Wild M3Z) or a Leitz, Ortholux II incident fluorescence microscope (excitation filter UG3, barrier filter K430/K460 and dichroic mirror TK 400), using UV light, respectively. Photographs were taken with Kodak Color, 100 ISO film.

Component analysis of resistance (experiment 2)

In this experiment, the plant lines pCH I and pNL II, and the rust lines rCH I and rNL II were used. Seeds were assigned randomly to peat pots (5 × 5 × 5 cm) filled with nutrient-amended peat (Floragard TKS 2). One seed was placed in a pot and 120 pots were used for each plant line. The experiment was conducted in a climate room with a 16 h light : 8 h dark photoperiod and a photosynthetically active radiation of 260 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Temperature was maintained at 23 ± 1°C in the day and 17 ± 1°C at night, with relative humidity (RH) of 30–60%. The developmental stage of plants was determined by counting the number of true leaves. Two categories of plants with either two or four leaves were selected at one day and inoculated with aeciospores using a settling tower. After inoculation, plants were enclosed in transparent plastic to retain humidity and placed in an incubation chamber for 15 h in the dark at 17°C. When the plastic was removed, plants were grown in a climate room under the conditions described above.

The first or second true leaf of plants was cut off 48 h after inoculation. A leaf disc of 0.28 cm^2 was punched out close to the margin of the lower left side of the leaf using a stainless steel cork borer. Afterwards, plants were placed back in the climate room. Fixing, clearing of pigments, and staining of the leaf discs for fluorescence microscopy were performed following Kuck et al. (1981). The number of the following clearly distinguishable infection structures were counted on each leaf disc: non-germinated spores (N_1), germinated spores (N_2), appressoria (N_3), penetration pegs (N_4), intra-epidermal vesicles (N_5), and primary hyphae, or incipient colonies (N_6).

The fractions of successive stages of rust development were computed as

$$[1] \quad \text{gsp/sp} = \frac{N_2 + N_3 + N_4 + N_5 + N_6}{N_1 + N_2 + N_3 + N_4 + N_5 + N_6}$$

$$[2] \quad \text{app/gsp} = \frac{N_3 + N_4 + N_5 + N_6}{N_2 + N_3 + N_4 + N_5 + N_6}$$

$$[3] \quad \text{peg/app} = \frac{N_4 + N_5 + N_6}{N_3 + N_4 + N_5 + N_6}$$

$$[4] \quad \text{ves/peg} = \frac{N_5 + N_6}{N_4 + N_5 + N_6}$$

$$[5] \quad \text{ph/ves} = \frac{N_6}{N_5 + N_6}$$

where gsp is germinated spores, sp is total number of spores, app is appressoria, peg is penetration pegs, ves is intra-epidermal vesicles, ph is primary hyphae, and N_1–N_6 are the numbers of the various infection structures as defined above.

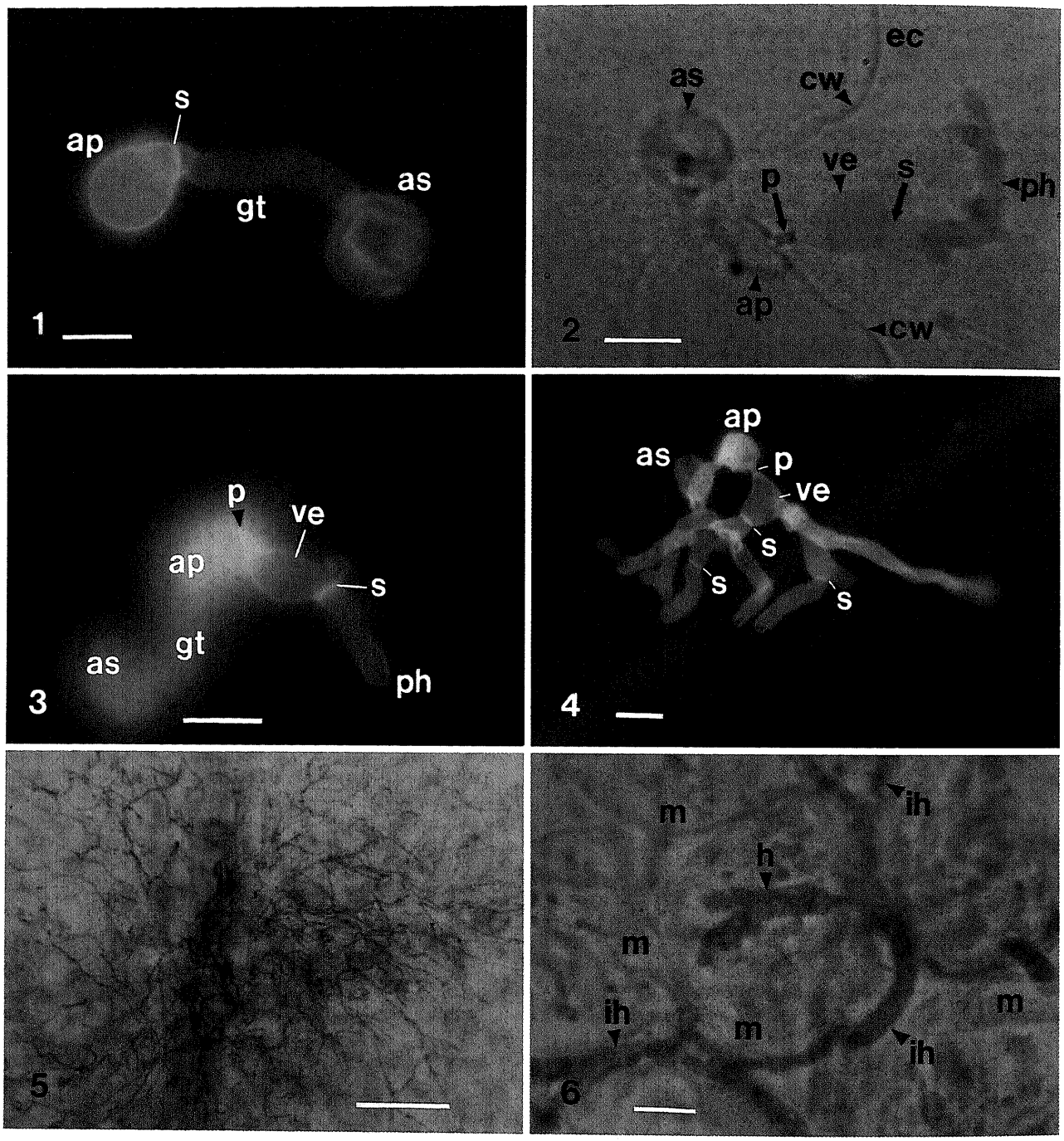
Ten days after inoculation when sori were open, the first or second leaf was cut off, and total leaf area and leaf area occupied by mycelium were measured using image analysis (Quantimet 500, Leica, Glattbrugg, Switzerland). Disease severity was assessed by calculating the fraction of leaf area occupied by mycelium.

The experiment was set up with 12 replicated plants and repeated three times (I–III). The experiments were analysed separately, as there were differences in test conditions between experiments. Data were subjected to a three-way analysis of variance with plant line (two categories), plant stage (two categories), and rust line (two categories) as the main factors and the various infection structures as the dependent variables. The mean values and standard errors were calculated for each transition of each plant line. Bartlett's test was used to test homogeneity of variance and the Scheffé F test to test differences among means. Variances were balanced, if required, with ln transformation. The nonparametric test of Kruskal–Wallis was used if transformation did not result in normality or homogeneity of variances (Sokal and Rohlf 1981). All statistical tests were performed on SPSS (Norusis 1990).

Host–pathogen interactions (experiment 3)

The plant lines pCH I, pNL II, and pUK and the rust lines rCH I, rNL II, and rUK were used to further detect specific interactions between plant and rust lines. The experiment was set up as described above, only disease severity was determined at 10 days af-

Figs. 1–6. Infection process of *P. lagenophorae* on *S. vulgaris* as seen with light (LM) and fluorescence (FM) microscopies. Fig. 1. Germinated aeciospore with appressorium on the leaf surface; FM. Scale bar = 10 μm. Fig. 2. Direct penetration into the epidermal cell at an epidermal cell junction and plugging the intra-epidermal vesicle with a glucan plug; LM. Scale bar = 10 μm. Fig. 3. Direct penetration and view on intra-epidermal infection structures at cell level; FM. Scale bar = 10 μm. Fig. 4. Young colony 48 h after inoculation (a.i.); FM. Scale bar = 10 μm. Fig. 5. Intercellular fungal growth. Colony at 6 days a.i.; LM. Scale bar = 50 μm. Fig. 6. M-Haustorium in a mesophyll cell; LM. Scale bar = 10 μm. as, aeciospore; gt, germ tube; ap, appressorium; s, septum; p, glucan plug; cw, cell wall; ec, epidermal cell; ve, intra-epidermal vesicle; ph, primary hypha; h, haustorium; ih, intercellular hypha; m, mesophyll cell.



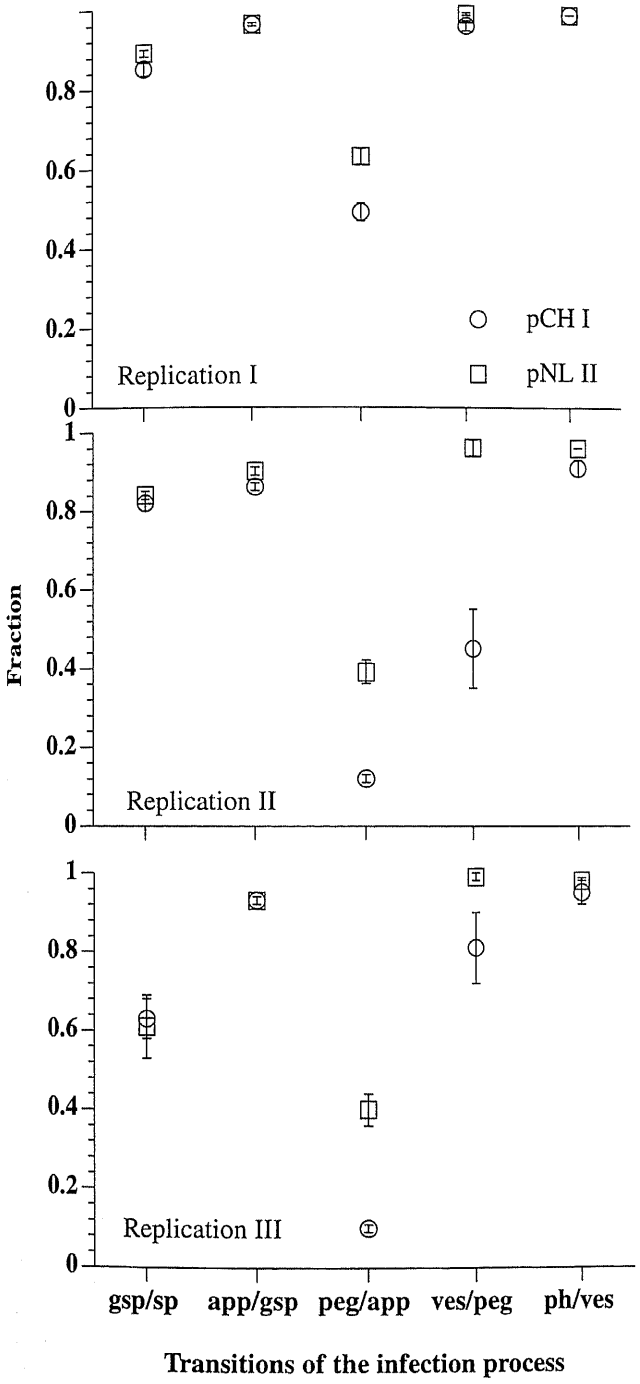
ter inoculation on both the whole first and second true leaf of the plant. The number of replicated plants was nine and the experiment was conducted once. The conditions in the climate room for photosynthetic active radiation was 130 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and relative humidity was 55–80%.

Results

Infection process of *P. lagenophorae*

Aeciospores were swollen and germinated within 4 h after inoculation (Fig. 1). A single, unbranched germ tube

Fig. 7. Quantification of the infection process of *P. lagenophorae* on plants of two *S. vulgaris* lines (experiment 2, replications I–III). Entries are mean fractions for two plant developmental stages and two rust lines per plant line each with 12 replicated plants (total 48 plants per plant line). Bars indicate standard error. Infection structures were analysed 48 h after inoculation on a leaf area of 0.28 cm^2. sp, total spores; gsp, germinated spores; app, appressoria; peg, penetration pegs; ves, intra-epidermal vesicles; ph, primary hyphae.



emerged from one of the germ pores. The germ tube elongated and swelled terminally to form an irregularly, ellipsoidal appressorium only slightly smaller than the spore from which it had arisen. A septum separated the appressorium from the germ tube. Appressoria present at 8 h after inoculation (a.i.) usually were positioned over the anticlinal walls of epidermal cell junctions but never over stomata (Fig. 2). The host was penetrated directly through the cuticle of the epidermal cell by an extremly short penetration peg. Some penetration pegs failed to enter the epidermal cell and ceased growing. Exceptionally, the rust penetrated through stomatal openings into the substomatal cavity. In these cases, germ tubes elongated and entered without morphological differentiation. This hypha continued intercellularly in the palisade layer and then stopped growing.

The small penetration peg elongated and plugged the neck of an intra-epidermal vesicle by a glucan plug (Xu and Mendgen 1991) at about 16 h a.i. (Figs. 2 and 3). For convenience, this stage will be referred to as penetration peg instead of glucan plug, when presenting and discussing the component analysis. At 24 h a.i., a primary hypha was present, which was separated by a septum from the intra-epidermal vesicle (Figs. 2 and 3). At 48 h a.i., the primary hypha was branched and septated (Fig. 4). Hyphae grew from the epidermal cell into the intercellular space in the palisade mesophyll. Colonies of the rust were visible at 6 days a.i. (Fig. 5). At 9 days a.i., aecia were produced and erupted through the upper epidermis.

Haustoria were visible 6 days a.i. within the cells located in the mesophyll as longish, fingerlike structures (Fig. 6). They were observed at low frequency, i.e., not every cell of a particular group of cells was involved. Haustorial mother cells could not be observed by fluorescence or light microscopy.

The following infection stages could be clearly distinguished: non-germinated spore (1), germinated spore (2), appressorium (3), penetration peg (glucan plug) (4), intra-epidermal vesicle (5), and primary hypha (6).

Component analysis of resistance

No major factor could be detected as influencing the total number of infection structures, ($\sum N_1 - N_6$) when assessed 48 h a.i. The analysis of variance (ANOVA) of replication I indicated significant effects of all three treatment factors ($P < 0.05$), but only plant line and rust line accounted for a significant amount of the variation in total number of infection structures in replications II and III, respectively. Less spores and subsequent infection structures were counted on plants of line pCH I (101.6 ± 7.3 spores per 0.28 cm^2; mean ± SE) than on plants of line pNL II (mean of 133.3 ± 8.0 spores per 0.28 cm^2; $P < 0.01$).

The lowest transition between infection stages was detected between the stages of appressorium and penetration peg (Fig. 7) indicating a bottleneck in the infection process. The factor plant line had a significant effect ($P < 0.001$) on that transition in all three replications (I–III) of experiment 2 (Table 1). The fraction of appressoria producing a penetration peg was in all three repeats significantly lower on plants of line pCH I than on pNL II plants (Table 2). After the establishment of penetration peg, further development of infection structures was likely.

Plant line also had a significant effect on disease severity in replications I and II (Table 3). In replication I, disease severity was higher on line pCH I than on pNL II (Table 4A). In contrast, disease severity was higher on pNL II than on

Table 1. Effects of treatment factors (two plant lines, two plant stages, and two rust lines) on the transition from appressorium to penetration peg (peg/app) of *P. lagenophorae* 48 h after inoculation (based on a leaf area of 0.28 cm^2; experiment 2).

Replication	Factor or interaction	df	*F*
I	Plant line (L)	1	25.94***
	Plant stage (S)	1	4.07*
	Rust line (R)	1	3.44ns
	L × S	1	<0.01ns
	L × R	1	2.75ns
	S × R	1	1.56ns
	L × R × S	1	<0.01ns
	Error	95	
II	L	1	99.81***
	S	1	3.85ns
	R	1	5.12*
	L × S	1	0.37ns
	L × R	1	1.08ns
	S × R	1	0.09ns
	L × R × S	1	2.62ns
	Error	86	
III	L	1	90.17***
	S	1	1.82ns
	R	1	1.70ns
	L × S	1	0.61ns
	L × R	1	0.21ns
	S × R	1	0.02ns
	L × R × S	1	3.11ns
	Error	91	

Note: The ANOVA for replication III was carried out on ln $(x + 0.5)$ transformed data. ns, not significant; *, $P < 0.05$; ***, $P < 0.001$.

pCH I in replications II and III. Plant stage had significant effects on disease severity in all three replications (Table 3). Plants inoculated at an early stage were less diseased than plants inoculated at an older stage (Table 4B). A significant effect of the rust line on disease severity was detected only in replication I (Table 3). Disease severity caused by rust line rCH I was less (mean fraction 0.31 ± 0.03) than disease severity caused by line rNL II (mean fraction 0.41 ± 0.02; $P < 0.001$).

Host–pathogen interactions

In experiment 3, plant line, plant stage, and rust line had significant effects on disease severity (Table 5). No significant interactions were detected. Plant line pNL II was the most susceptible with respect to all three rust lines tested (Table 6). *Puccinia lagenophorae* line rNL II was the most aggressive, irrespective of the host plant concerned. Disease severity was higher at older (mean fraction 0.23) than at younger plant stages (mean fraction 0.19).

Discussion

Infection process

Appressoria of *P. lagenophorae* attached preferentially near or at epidermal cell junctions such as it has been shown for appressoria formed by basidiospores (Gold and Mendgen 1984) and by uredospores of *Physopella zeae* (Mains) Cumm. & Ramachar (Hunt 1968). Cutler et al. (1982) assumed greater availability of nutrients and moisture at cell junctions because of a higher rate of exosmosis and (or) chemical composition and the physical structure of wax layer that affects both pH and wettability of the leaf surface.

Table 2. The effect of plant line on the transition from appressorium to penetration peg (peg/app) of *P. lagenophorae* as assessed 48 h after inoculation on a leaf area of 0.28 cm^2 (experiment 2).

Replication	Plant line	peg/app (mean ± SE)
I	pCH I	0.49±0.02***
	pNL II	0.63±0.02
II	pCH I	0.12±0.01***
	pNL II	0.39±0.03
III	pCH I	0.10±0.01***
	pNL II	0.40±0.04

***Significant difference among the plant lines ($P < 0.001$, ANOVA).

Table 3. The effects of the treatment factors (two plant lines, two plant stages, and two rust lines) on disease severity of *P. lagenophorae* on the first or second true leaf assessed on *S. vulgaris* 10 days after inoculation (experiment 2).

Replication	Factor or interaction	df	*F*
I	Plant line (L)	1	8.69**
	Plant stage (S)	1	39.67***
	Rust line (R)	1	10.92***
	L × S	1	1.82ns
	L × R	1	1.40ns
	S × R	1	3.31ns
	L × R × S	1	0.12ns
	Error	95	
II	L	1	11.15***
	S	1	55.76***
	R	1	0.13ns
	L × S	1	0.98ns
	L × R	1	1.03ns
	S × R	1	0.33ns
	L × R × S	1	2.96ns
	Error	86	
III	L	1	3.20ns
	S	1	18.15***
	R	1	1.86ns
	L × S	1	0.16ns
	L × R	1	0.02ns
	S × R	1	0.70ns
	L × R × S	1	0.57ns
	Error	94	

Note: Disease severity is defined as leaf area occupied by mycelium. ns, not significant; **, $P < 0.01$; ***, $P < 0.001$.

The life cycle of *P. lagenophorae* on *S. vulgaris* has not yet been understood completely and the nuclear conditions remain to be studied. Based on the initial assumption that this study has been carried out with the dikaryotic stage of infection (aecial stage; Baka and Lösel 1992), the observed direct penetration of aeciospores of *P. lagenophorae* is rather unusual. Direct penetration of the leaf cuticle by means of germinated aeciospores has been reported only for *Arthuromyces peckianus* (E. Howe) Cummins & Y. Hirutsaka (syn. *Gymnoconia interstitialis* (Schl.) Lagerh.) on *Rubus* spp. (Pady 1935). Unfortunately, no results are available on the infection process of this rust on its native host

Table 4. The effects of plant line and plant stage on disease severity of *P. lagenophorae* (experiment 2).

(A) Plant line.		
Replication	Plant line	Disease severity[a] (mean ± SE)
I	pCH I	0.40±0.03**
	pNL II	0.32±0.02
II	pCH I	0.19±0.03***
	pNL II	0.32±0.03
III	pCH I	0.16±0.02ns
	pNL II	0.21±0.02
(B) Plant stage.		
Replication	Plant stage[b]	Disease severity[a] (mean ± SE)
I	2	0.27±0.03***
	4	0.45±0.02
II	2	0.12±0.02***
	4	0.37±0.02
III	2	0.13±0.02***
	4	0.24±0.02

[a]Disease severity is defined as the leaf area occupied by mycelium. ns, not significant; **, $P < 0.01$; ***, $P < 0.001$.
[b]Plant stage was expressed as two leaf stage (2) or four leaf stage (4).

Table 5. Influence of plant line, plant stage, rust line, and their interactions on disease severity[a] of *P. lagenophorae* measured 10 days after inoculation (experiment 3).

Factor and interaction	df	F^b
Plant line (L)	2	21.87***
Rust line (R)	2	7.77***
Plant stage (S)	1	4.71*
L × S	2	2.02ns
L × R	4	1.66ns
S × R	2	0.06ns
L × S × R	4	0.29ns
Error	143	

[a]Disease severity is defined as the leaf area occupied by mycelium.
[b]ns, not significant; *, $P < 0.05$; ***, $P < 0.001$.

Table 6. Disease severity on the *S. vulgaris* lines pCH I, pNL II, and pUK infected by three different rust lines of *P. lagenophorae* (rCH, rNL II, rUK).

	rUK	rCH I	rNL II	Mean ± SE
pCH I	0.14±0.02	0.13±0.02	0.21±0.03	0.16±0.01
pUK	0.17±0.03	0.15±0.03	0.23±0.03	0.17±0.01
pNL II	0.22±0.03	0.32±0.02	0.33±0.02	0.27±0.02
Mean ± SE	0.18±0.02	0.20±0.02	0.26±0.02	0.21

Note: Disease severity is defined as the leaf area occupied by mycelium. Values are means of the fraction of leaf area occupied by mycelium ± SE of 18 replicated plants.

Lagenophora billardieri Cass. in Australia (Wilson et al. 1965).

The morphological features of the investigated infection, such as the small penetration peg and the direct penetration are all typical of the basidiospore-derived monokaryotic parasitic stage of other rust fungi (Xu and Mendgen 1991). Moreover, the haustorial mother cells were not clearly seen indicating again characteristics of the monokaryotic parasitic stage, where haustorial mother cells are not well differentiated as they are in the dikaryotic stage (Harder and Chong 1984).

In many rusts the haustorium formation occurs within 24 h a.i. (e.g., Stark-Urnau and Mendgen 1993), and one or two haustoria are developed in each cell relative to the area of the colony (Kneal and Farrar 1985). This is in contrast to *P. lagenophorae* where haustoria developed only between 3 and 6 days after inoculation and at low frequency. The fingerlike shapes of the observed haustoria corresponded with the so called M-haustoria, typical for monokaryotic parasitic stages, detected and reported by Baka and Lösel (1992). They investigated especially late infection of vascular tissues of *S. vulgaris* by *P. lagenophorae*.

Component analysis of resistance

Low infectivity of *S. vulgaris* plants by the rust can be attributed mainly to a reduced formation of penetration pegs. It seems likely that the inhibition of penetration peg formation is the most important mechanism of resistance in the infection process considering the stages investigated. The generally low transition in all three experiments and its dependence on plant line suggests that defence mechanisms are effective near the leaf surface, i.e., in or at the epidermal cell.

Differences in resistance between plant lines (experiments 2 and 3) may be due to anatomical or morphological differences. Russell (1975) reported that a dependence on leaf-angle orientation resulted in a significant difference in the amount of *P. striiformis* spores deposited. Moreover, Niks (1983) attributed differences in plant lines to differences in leaf surface (e.g., hardness, hairiness, or composition of wax layer).

Resistance was localized at different phases of the infection process: (*i*) at spore adhesion, (*ii*) until the formation of primary hyphae, and (*iii*) between the formation of primary hyphae and sori. Spore adhesion, however, raises questions because differences in resistance could be due to nonuniform spore application. Therefore, resistance might be related to two different mechanisms, although pleiotropic effects cannot be ruled out.

This study clearly showed that *S. vulgaris* leaves are increasingly susceptible to disease with age. A similar observation was made with *Rumex crispus* L. and *Rumex obtusifolius* L. infected with *Uromyces rumicis* (Schum.) Wint. (Hatcher et al. 1995). This contradicts findings with regard to infection structure and uredosori development of *Hemileia vastatrix* Berk. & Broome on *Coffea* genotypes (Coutinho et al. 1994). In general, effects of plant stage may vary in expression and seem to depend on the particular host–pathogen interaction and obviously on the environment where the interaction occurs.

Our results show the use of component analysis as a comprehensive approach for comparisons of plant lines and plant stages to assess resistance. Component analysis may provide also a tool for (*i*) carrying out host-specificity tests that are fundamental for the assessment of biological control agents, (*ii*) the evaluation of the influence of pesticides and adjuvants on the compatibility of the biocontrol agent, and (*iii*) the influence of secondary necrotrophs on the enhancement of biological control agents.

Host–pathogen interactions

Results from experiment 2 were confirmed by experiment 3 where a wider range of geographical sites from central Europe to the United Kingdom was included. Disease severity showed a continuous range of variation in resistance to *P. lagenophorae*. Plant line pNL II usually was the most susceptible to all rust lines tested with rNL II being the most aggressive. Significant differential interactions suggesting the existence of a gene-for-gene relationship between host resistance genes and pathogen virulence genes were not found in this study. Therefore, the results indicate expression of resistance to be race-nonspecific and quantitative (sensu Parlevliet 1981, 1989) in this weed pathosystem.

Occurrence of race-specific resistance in wild and weed pathosystems is now sufficiently well documented and race-specific resistance seems to be widespread in many plant species. The relatively old weed–pathogen association *S. vulgaris – Erysiphe fischeri* Blumer provided evidence for the existence of both completely and partially expressed resistance (Bevan et al. 1993*a*) that is either race-specific or race-nonspecific (Bevan et al. 1993*b*; Harry and Clarke 1986). In this association the gene-for-gene theory might be applicable. In this context, Clarke (1997) assumed that race-specific resistance might not be expected in a recently established association such as *S. vulgaris – P. lagenophorae*.

Quantitative characters such as aggressiveness of the pathogen and quantitative resistance in the host plant are likely to be affected by environmental variations (Kulkarni and Chopra 1982). Environmental factors such as temperature, humidity, or light intensity might be responsible for the variation of resistance levels among our replications, such as the higher susceptibility on the Swiss plant line in experiment 2, replication I (when compared with replications II and III; Table 4). In many cases, however, influences of environmental stress on disease susceptibility are usually found during fungal development within the host plant. Paul and Ayres (1984) demonstrated that rusted *S. vulgaris* plants and additional drought stress resulted in inhibition of leaf growth, but not in inhibition of fungal development.

Implications for biological control

Complete resistance (qualitative resistance) will prevent multiplication of the pathogen. In the case of quantitative resistance, host impact and spore production may still occur. In none of the interactions analysed, however, was host–pathogen incompatibility found. Quantitative resistance is considered to be durable if it remains effective during prolonged and widespread use of the pathogen in an environment favourable to the disease (Johnson 1984). Unfortunately, little is known about sexual reproduction and genetic variation present in *P. lagenophorae* in Europe. Great genotypic variability might not be expected because of the recent introduction of the rust in central Europe and the United Kingdom. Our results, however, demonstrate an increase of the level of disease on individual genotypes by the more aggressive rust line rNL II. Assuming a conducive environment, greater severity of infection may result in larger spore production and better stimulation of a disease epidemics (Frantzen and Müller-Schärer 1998).

On a long-term basis, consequences of differences in the level of disease on individual plants and selection by the more aggressive pathogens will favour the buildup of less susceptible weed populations (Burdon 1991). Sustainability of biological weed control would then be reduced. Other factors influencing the fitness of *S. vulgaris* plants may, however, alleviate or override the effects imposed by the rust fungus (Burdon 1987). In this context, it will be most important to define the level of disease that might be necessary to sufficiently impact the host plant and its influence on the speed of the epidemic.

Acknowledgements

We are grateful to Jos Frantzen for helpful discussions and statistical advice; to Matthias Hahn, Paul Hatcher, R. Charudattan, and Margareth Smither-Kopperl for critical reading of the manuscript; and to two anonymous reviewers for helpful suggestions. The project was part of the European COST Project No. 816. We thank the Swiss Ministry of Education and Science and the Swiss Federal Research Station for Fruit-Growing, Horticulture and Viticulture, Wädenswil, for their financial support.

References

Baka, Z.A., and Lösel, D.M. 1992. Infection of vascular tissues by the autoecious rusts *Puccinia punctiformis* and *Puccinia lagenophorae*: a cytological study. Physiol. Mol. Plant Pathol. **40**: 411–421.

Bevan, J.R., Crute, I.R., and Clarke, D.D. 1993*a*. Diversity and variation in expression of resistance to *Erysiphe fischeri* in *Senecio vulgaris*. Plant Pathol. **42**: 647–653.

Bevan, J.R., Crute, I.R., and Clarke, D.D. 1993*b*. Variation for virulence in *Erysiphe fischeri* from *Senecio vulgaris*. Plant Pathol. **42**: 622–635.

Bruzzese, E., and Hasan, S. 1983. A whole leaf clearing and staining technique for host specificity studies of rust fungi. Plant Pathol. **32**: 335–338.

Burdon, J.J. 1987. Diseases and plant population biology. Cambridge University Press, Cambridge, U.K.

Burdon, J.J. 1991. Fungal pathogens as selective forces in plant populations and communities. Aust. J. Ecol. **16**: 423–432.

Charudattan, R. 1988. Inundative control of weeds with indigenous fungal pathogens. *In* Fungi in biological control systems. *Edited by* M.N. Burge. Manchester University Press, Manchester, U.K. pp. 86–110.

Clarke, D.D. 1997. The genetic structure of natural pathosystems. *In* The gene-for-gene relationship in plant–parasite interactions. *Edited by* I.R. Crute, E.B. Holub, and J.J. Burdon. CAB International, Wallingford, U.K. pp. 231–243.

Coutinho, T.A., Rijkenberg, F.H.J., and Van Asch, M.A.J. 1994. The effect of leaf age on infection of *Coffea* genotypes by *Hemileia vastatrix*. Plant Pathol. **43**: 97–103.

Cutler, D.F., Alvin, K.L., and Price, C.E. 1982. The plant cuticle. Academic Press, London.

Dinoor, A., and Eshed, N. 1984. The role and importance of pathogens in natural plant communities. Annu. Rev. Phytopathol. **22**: 443–466.

Ennos, R. 1992. Ecological genetics of parasitism. *In* Genes in ecology. *Edited by* R.J. Berry, T.J. Crawford, and G.M. Hewitt. Blackwell Scientific Publications, London. pp. 255–279.

Frantzen, J., and Hatcher, P.E. 1997. A fresh view on the control of the annual plant *Senecio vulgaris*. Integr. Pest Manage. Rev. **2**: 77–85.

Frantzen, J., and Müller-Schärer, H. 1998. A theory relating focal epidemics to crop–weed interactions. Phytopathology, **88**: 180–184.

Gold, R.E., and Mendgen, K. 1984. Cytology of basidiospore germination, penetration, and early colonization of *Phaseolus vulgaris* by *Uromyces appendiculatus* var. *appendiculatus*. Can. J. Bot. **62**: 1989–2002.

Harder, D.E., and Chong, J. 1984. Structure and physiology of haustoria. *In* The cereal rusts: origins, specificity, structure, and physiology. *Edited by* W.R. Bushnell and A.P. Roelfs. Academic Press, Inc., London. pp. 431–476.

Harry, I.B., and Clarke, D.D. 1986. Race-specific resistance in groundsel (*Senecio vulgaris*) to the powdery mildew *Erysiphe fischeri*. New Phytol. **103**: 167–175.

Hatcher, P.E., Ayres, P.G., and Paul, N.D. 1995. The effect of natural and simulated insect herbivory, and leaf age, on the process of infection of *Rumex crispus* L., and *R. obtusifolius* L. by *Uromyces rumicis* (Schum.) Wint. New Phytol. **130**: 239–249.

Holm, L., Doll, J., Holm, E., Pancho, J., and Herberger, J. 1997. World weeds. Natural histories and distrbution. John Wiley & Sons, New York. pp. 740–750.

Holt, J.S., and LeBaron, H.M. 1990. Significance and distribution of herbicide resistance. Weed Technol. **4**: 141–149.

Hunt, P. 1968. Cuticular penetration by germinating uredospores. Trans. Br. Mycol. Soc. **51**: 103–112.

Jacobs, A.S., Pretorius, Z.A., Kloppers, F.J., and Cox, T.S. 1996. Mechanisms associated with wheat leaf rust resistance derived from *Triticum monococcum*. Phytopathology, **86**: 588–595.

Johnson, R. 1984. A critical analysis of durable resistance. Annu. Rev. Phytopathol. **22**: 309–330.

Kadereit, J.W. 1984. The origin of *Senecio vulgaris* (*Asteraceae*). Plant Syst. Evol. **145**: 135–153.

Kavanagh, T. 1964. A new rust species of groundsel in Ireland. Ir. Nat. J. **14**: 214.

Kneale, J., and Farrar, J.F. 1985. The localization and frequency of haustoria in colonies of brown rust on barley leaves. New Phytol. **101**: 495–505.

Kuck, K.H., Tiburzy, R., Hänssler, G., and Reisener, H.-J. 1981. Visualization of rust haustoria in wheat leaves by using fluorochromes. Physiol. Plant Pathol. **19**: 439–441.

Kulkarni, R.N., and Chopra, V.L. 1982. Environment as the cause of differential interaction between host cultivars and pathogenic races. Phytopathology, **72**: 1384–1386.

Müller-Schärer, H., and Frantzen, J. 1996. An emerging system management approach for biological weed control in crops: *Senecio vulgaris* as a research model. Weed Res. **36**: 483–491.

Müller-Schärer, H., and Rieger, S. 1998. Epidemic spread of the rust fungus *Puccinia lagenophorae* and its impact on the competitive ability of *Senecio vulgaris* in celeriac during early development. Biocontr. Sci. Technol. **8**: 59–72.

Müller-Schärer, H., and Wyss, G.S. 1994. Das Gemeine Kreuzkraut (*Senecio vulgaris* L.): Problemunkraut und Möglichkeiten der biologischen Bekämpfung. Z. Pflanzenkr. Pflanzenschutz Sonderh. **XIV**: 201–209.

Niks, R.E. 1983. Studies on the histology of partial resistance in barley to leafrust, *Puccinia hordei*. Ph.D. thesis, Institute of Plant Breeding, Agricultural University, Wageningen, the Netherlands.

Norusis, M.J. 1990. SPSS 6.1 guide to data analysis. Prentice-Hall, Englewood Cliffs, N.J.

Omar, M.B., Bolland, L., and Heather, W.A. 1978. A permanent mounting medium for fungi. Stain Technol. **53**: 293–294.

Pady, S.M. 1935. Aeciospore infection in *Gymnoconia interstitialis* by penetration of the cuticle. Phytopathology, **25**: 453–474.

Parlevliet, J.E. 1981. Race-non-specific disease resistance. *In* Strategies for the control of cereal disease. *Edited by* J.F. Jenkyn and R.T. Plumb. Blackwell Scientific Publications, Oxford, U.K. pp. 47–54.

Parlevliet, J.E. 1989. Identification and evaluation of quantitative resistance. *In* Plant disease epidemiology. *Edited by* K.J. Leonard and W.E. Fry. McGraw-Hill Publishing Company, New York. pp. 215–248.

Paul, N.D., and Ayres, P.G. 1984. Effects of rust and post-infection drought on photosynthesis, growth and water relations in groundsel. Plant Pathol. **33**: 561–569.

Paul, N.D., and Ayres, P.G. 1987. Effects of rust infection of *Senecio vulgaris* on competition with lettuce. Weed Res. **27**: 431–441.

Paul, N.D., Ayres, P.G., and Hallett, S.G. 1993. Mycoherbicides and other biocontrol agents for *Senecio* spp. Pestic. Sci. **37**: 323–329.

Robinson, R.A. 1976. Plant pathosystems. Springer-Verlag, Berlin.

Rohringer, R., Kim, W.K., Samborski, D.J., and Howes, N.K. 1977. Calcofluor: an optical brightener for fluorescence microscopy of fungal plant parasites in leaves. Phytopathology, **67**: 808–810.

Russell, G.E. 1975. Deposition of *Puccinia stiiformis* uredospores on adult wheat plants in laboratory experiments. Cereal Rusts Bull. **3**: 40–43.

Scholler, M. 1993. *Puccinia lagenophorae*, a rust fungus originating from Australia, now found in Mecklenburg-Vorpommern. Zentralbl. Mikrobiol. **148**: 223–228.

Scholler, M. 1997. Rust fungi on *Bellis perennis* in Central Europe: delimination and distribution. Sydowia, **49**: 174–181.

Sokal, R.R., and Rohlf, F.J. 1981. Biometry. W.H. Freeman & Co., New York.

Stark-Urnau, M., and Mendgen, K. 1993. Differentiation of aecidiospore- and uredospore-derived infection structures on cowpea leaves and on artificial surfaces by *Uromyces vignae*. Can. J. Bot. **71**: 1236–1242.

Viennot-Bourgin, G. 1964. La Rouille australienne du Séneçon. Rev. Mycol. **29**: 241–258.

Watson, A.K. 1991. The classical approach with plant pathogens. *In* Microbial control of weeds. *Edited by* D.O. TeBeest. Chapman & Hall, New York. pp. 3–23.

Wilson, I.M., Walshaw, D.F., and Walker, J. 1965. The new groundsel rust in Britain and its relationship to certain Australasian rusts. Trans. Br. Mycol. Soc. **48**: 501–511.

Xu, H., and Mendgen, K. 1991. Early events in living epidermal cells of cowpea and broad bean during infection with basidiospores of the cowpea rust fungus. Can. J. Bot. **69**: 2279–2285.

Zadoks, J.C. 1972. Modern concepts of disease resistance in cereals. *In* The Way Ahead in Plant Breeding. Proceedings of the 6th Congress of Eucarpia, 29 June – 2 July 1971, Cambridge, U.K. *Edited by* F.A.G.H. Lupton, G. Jenkins, and R. Johnson. Adlard & Sons Ltd., Bartholomew Press, Dorking, U.K. pp. 89–98.

Zadoks, J.C., and Schein, R.D. 1979. Epidemiology and plant disease management. Oxford University Press, Inc., Oxford, U.K.