

## Host Range of the Haplontic Phase of *Uromyces rumicis*

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### Abstract

Teliospores of *Uromyces rumicis*, produced on detached dock leaves, could be activated to germinate by storing the spores for 60 days on water agar at 2°C in continuous darkness. Within 10 days, after changing the teliospores to 8°C under light, up to approximately 70% of the teliospores germinated with basidia and basidiospores. *Ranunculus Ficaria* was the only species that could be infected by the basidiospores (haplontic phase) of *U. rumicis*. Eleven species of the genus *Ranunculus*, 16 other species out of the family Ranunculaceae, 12 crop plants, *Rumex crispus*, *R. obtusifolius* and *Berberis vulgaris* could not be infected. Lack of knowledge about the host range of the haplontic phase of *U. rumicis* is no more a prevention of biological control of *Rumex* weeds with this rust fungus.

### L'Éventail de Plantes-Hôtes de la Phase Haplontique d'*Uromyces rumicis*

Des téliospores d'*Uromyces rumicis*, élevés sur des feuilles détachées de patience (*Rumex patientia*), et tenus sur le gel d'agar à 2°C, dans l'obscurité totale, ont pu être activées à la germination. A moins de 10 jours d'un changement de conditions (8°C, et lumière), 70% environ des téliospores avaient germé, avec des basidia et des basidiospores. Le *Ranunculus ficaria* était la seule espèce pouvant être infectée par les basidiospores (phase haplontique) d'*U. rumicis*. Onze espèces du genre *Ranunculus*, 16 autres espèces de la famille Ranunculacées, 12 plantes vivières, *Rumex crispus*, *R. obtusifolius* et *Berberis vulgaris* n'ont pu être infectées. Le manque de connaissance concernant l'éventail des hôtes d'*U. rumicis* n'empêche plus son emploi dans le biocontrôle des *Rumex* nuisibles.

### Introduction

In 1967, the U.S. Department of Agriculture began to study the potential of *Uromyces rumicis* (Schum.) Wint. (Uredinales) as a biological control agent against *Rumex crispus* L. (curly dock; Polygonaceae). *R. crispus* is a common weed in the United States and in other regions of the world.

*U. rumicis* is a macrocyclic heteroecious rust fungus. In the dicaryotic phase it infects species of the subgenus *Lapathum* within the genus *Rumex* (Gäumann 1959; Inman 1971). This subgenus includes common weeds like *R. crispus*, *R. obtusifolius* L., and *R. pulcher* L. Besides this subgenus, Morris (1982) described the fungus on *Emex australis* Steinh. (Polygonaceae) in South Africa. The risk of infecting economic plants in the dicaryotic phase is presumed by Inman (1971) as sufficiently low.

Less is known concerning the host range of the haplontic phase of *U. rumicis*. Tranzschel (1905, 1909) and Gäumann (1931) described *Ranunculus Ficaria* L. (Ranunculaceae) as the only alternate host of *U. rumicis*. Inman (1971) was unsuccessful in confirming these results, because of inability of teliospores to germinate.

The rust is presumed to be absent from the United States and is not listed by Arthur and Cummins (1962) as occurring in the country (cit. in Inman 1971). The sufficient

potential of the rust in controlling curly dock was demonstrated by Inman (1971). Nevertheless a release of the fungus in the United States was prevented, because of a failure in testing the host range of the haplontic phase of *U. rumicis* (Frank 1973).

The purpose of this paper is to present a method to activate teliospore germination under controlled conditions and to test several selected species of plants for susceptibility to *U. rumicis* in the haplontic phase.

Table 1. List of crops plants, other plants and Ranunculaceae (except genus *Ranunculus*) tested.

Species	Cultivar	Notes <sup>1</sup>
Crop plants		
<i>Triticum aestivum</i> L.	Kolibri	b
	Probus	b
<i>Zea mays</i> L.	Orla 312	b
	LG 11	b
<i>Beta vulgaris</i> L.	Kawemono	d
<i>Spinacia oleracea</i> L.	Monapa	a
<i>Medicago sativa</i> L.		e
<i>Phaseolus vulgaris</i> L.	Top crop	a
<i>Pisum sativum</i> L.	Eldo	a
<i>Trifolium pratense</i> L.		e
<i>T. repens</i> L.		e
<i>Nicotiana tabacum</i> L.	Sota 50	c
<i>Solanum lycopersicum</i> L.	Montfavit	a
<i>Cucumis sativus</i> L.	Chin. Schlange	a
Ranunculaceae		
<i>Aconitum compactum</i> Rchb.		f *
<i>Anemone blanda</i> Sch. et K.	Pink Star	a *
<i>A. coronaria</i> L.	de Caen	a *
<i>A. pulsatilla</i> L.	Küchenschelle	a
<i>Aquilegia alpina</i> L.	alpine Columbine blue	a
<i>A. hybrida</i> L.	Koralle lachsrot	a *
<i>Caltha palustris</i> L.		f *
<i>Clematis Muraski</i>	Deep purple	a *
<i>Delphinium ajacis</i> L.	Kalsey	a
<i>D. cultorum</i> Voss.	Stand up	a *
<i>D. grandiflorum</i> L.	Chinese	a
<i>D. zalil (sulphureum)</i>		
Aitch. et Hensl.	Rittersporn	a
<i>Eranthis hiemalis</i> (L.)		
Salisb.	Winterling	a *
<i>Nigella damascena</i> L.	Miss Jeckyll	a
<i>Thalictrum dipterocarpum</i>		
Franch.	purpurlila	a *
<i>Trollius europaeus</i> L.	Globe Flower	a *
Other Plants		
<i>Berberis vulgaris</i> L.		f
<i>Rumex crispus</i> L.		f *
<i>R. obtusifolius</i> L.		f *

<sup>1</sup> \* = Species tested in the climatic chamber and in the field experiment; a = Samen Mauser, Zurich, Switzerland; b = UFA Samen, Winterthur, Switzerland; c = Sota, Changins, Switzerland; d = Kleinwanzlebner Saatzzucht, Kleinwanzleben, Western Germany; e = Landwirtschaftliche Forschungsanstalt, Reckenholz Zurich; and f = collected in Switzerland by us.

## Materials and Methods

### *Rust and Plant Materials*

Uredospores of *U. rumicis* were collected in Switzerland: in Männedorf (R-113) on *R. crispus* and in Mols (R-35) on *R. obtusifolius*. Both strains were collected by the authors and propagated in the greenhouse on their original hosts. Plant material used is listed in Tables 1 and 2. Plants were either collected by us or obtained (see Tables 1 and 2) and were grown in sterilized soil (Potgrond Topferde, de Baat, Netherlands) in clay pots (diam. 12 cm) in the greenhouse (16 h light at 20°C, 8 h dark at 15°C, 60% r.h.).

Table 2. List of species of the genus *Ranunculus* tested.

Species	Origin	Notes <sup>1</sup>
<i>R. aconitifolius</i> L.	Wängialp, Switzerland	*
<i>R. alpestris</i> L.	Frutt, Switzerland	a
<i>R. arvensis</i> L.	Jaca, Spain	d
<i>R. auricomus</i> L. <i>s.l.</i>	Affoltern, Switzerland	*
<i>R. californicus</i> Benth.	USA	c
<i>R. Ficaria</i> L.	Männedorf, Switzerland	
	Grüningen, Switzerland	*
	Luzern, Switzerland	*
<i>R. flammula</i> L.	Emmen, Switzerland	a
<i>R. Friesianus</i> Jordan	Klöntalersee, Switzerland	
	Bern, Switzerland	*
<i>R. gramineus</i> L.	Switzerland	a
<i>R. millefolius</i> Banks & Sol.	Greece	a
<i>R. repens</i> L.	Zurich, Switzerland	*
<i>Ranunculus</i> sp.	commercial seed	b *

<sup>1</sup> \* = species tested in the climatic chamber and in the field experiment. Seeds or plants obtained by: a = garden center "Alpengarten Eschmann", Emmen Switzerland; b = Samen Mauser, Zurich, Switzerland; c = University of California, Santa Barbara, USA; d = Botanical Garden of Basel, Switzerland. All other *Ranunculus* species were collected by the authors.

### *Method to Activate Germination of Teliospores*

Teliospores were produced on detached leaves of *R. obtusifolius*. Young leaves were cut, inoculated with a uredospore suspension and put in a pot filled with Perlit® (granular mineral of quartz expanded by heating) and tap water. Leaves were covered with a plastic bag and put in a chamber at 8°C. Artificial illumination was given for 12 h (Philips, TL 20W/55, 1500–2500 lux). Approximately 35–45 days after inoculation the teliospores, formed on the detached leaves, were suspended in water. The suspension was passed through a hydrophilic cellulose nitrate filter (Sartorius 8 µ, diam. 5 cm; Gold and Mendgen 1983). The filter with the thin spore layer was put into one petri dish containing 2% water agar (Bacto-Agar, Difco).

In experiment A, teliospores in petri dishes were incubated at different temperatures (2, 8, 15, 21°C) under artificial illumination (12 h) or in continuous dark. Every 10 days the percent of germinated teliospores was determined (10 × 100 teliospores were scored) microscopically in each treatment. A teliospore was considered to be germinated if the germ tube (the basidium) was at least longer than the spore.

In experiment B, teliospores were stored for 60 days at 2°C in continuous dark. After this storage, spores on filters were changed to different temperatures (2, 8, 15, 21°C). Artificial illumination was given for 12 h. Percent of germinated teliospores was determined 10 days later, as described above.

Experiments A and B were done with R-35. For each treatment, three petri dishes were used. Every experiment was repeated at least three times.

Results of germination tests were analysed using Duncan test at the level  $P < 0.05$ .

#### *Inoculation Experiment*

Teliospores were produced and put on cellulose nitrate filters as described above. Spores were then stored 60 days at 2°C in the dark. After that treatment they were suspended in water and sprayed on the different test plants (Tables 1 and 2). At the moment of inoculation all plants were in a young, growing stage. Plants were placed for 2 wks in a plastic chamber (12 h light at 12°C, 12 h dark at 10°C, 100% r.h.). After removing the plastic, r.h. was dropped to 80%.

All *Ranunculus* species were inoculated separately with each strain (R-35 and R-113). Other species were inoculated with a mixture of the two strains. All inoculation experiments were done with at least five plants/species or ecotype and were repeated three times. Infection severity was checked one and two months after inoculation.

#### *Field Experiment*

During three years, different species of Ranunculaceae (see Tables 1 and 2) were growing together with *R. crispus* and *R. obtusifolius* in a field plot (10 m<sup>2</sup>). Alternately a row of *Rumex* plants were arranged next to a row of Ranunculaceae plants. At least four plants of each species of Ranunculaceae were used. Each summer the docks were inoculated with uredospores of different strains. In spring, plants were checked for aecia.

### **Results**

#### *Teliospore Formation*

Teliospore formation occurred 20–25 days following inoculation of the detached dock leaves with uredospores. Rust strain R-35 produced approximately 80% teliospores, and strain R-113 up to 40–50% teliospores (the rest were uredospores).

#### *Teliospore Germination*

Teliospores collected in nature or in the greenhouse germinated after winter storage outdoors. In spring (March to April), basidia and basidiospores were observed.

Teliospores, produced on detached leaves, germinated with basidia and basidiospores after relatively short storage on filters on water agar. In experiment A (Fig. 1) the best germination rate of teliospores was observed during an incubation at 2°C under light. They began to germinate abundantly after 50 days. Thirty days later up to 60% had germinated. At the same temperature but in continuous dark, beginning of germination was 10 days later, and germination rate remained less at any time. An incubation at 8°C under light or in continuous darkness had also a smaller effect on germination. During incubation at 15 or 21°C under light or in continuous dark the teliospores did not germinate.

In experiment B (Fig. 2), germination rate of teliospores stored for 60 days at 2°C in continuous dark, could be increased by incubating spores at 8 or 15°C under light.

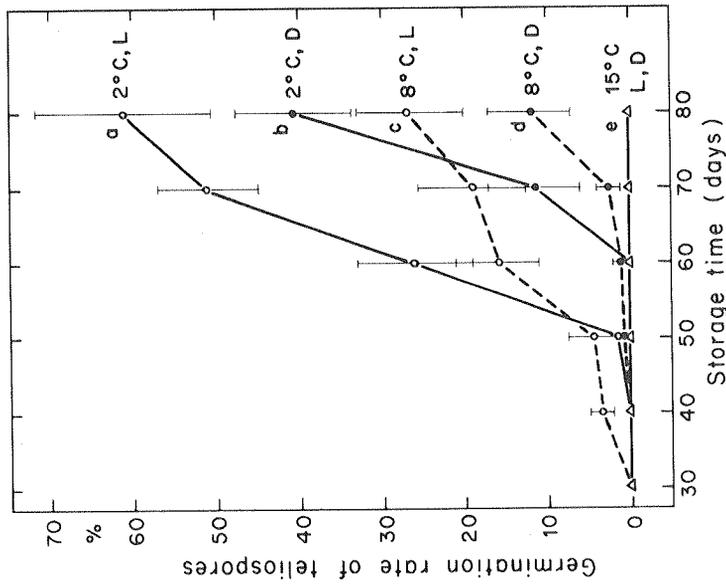
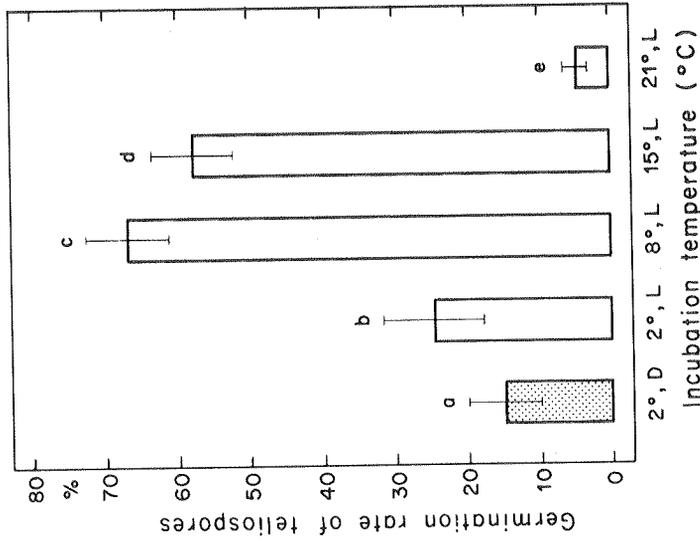


Fig. 1. (Left) Experiment A: Effect of incubation at different temperatures under light or in continuous darkness on germination rate of teliospores of *Uromyces rumicis* (Schum.) Wint. Points with a different letter are significantly different ( $P < 0.05$ ). D = continuous darkness, L = 12 h light.

Fig. 2. (Right) Experiment B: Effect of different temperatures on germination rate of teliospores of *Uromyces rumicis* (Schum.) Wint. stored for 60 days at 2°C in the dark. Germination rate was determined after 10 days of incubation. Columns with a different letter are significantly different ( $P < 0.05$ ). D = continuous darkness, L = 12 h light.

Within 10 days at 8°C up to approximately 70% of teliospores germinated; a similar effect was achieved at 15°C. Remaining at 2°C in continuous dark, or incubating at 2 or 21°C under light, had less effect on the germination rate.

Teliospores of rust strains R-35 and R-113, used for the inoculation experiment, both germinated up to 70%.

#### *Host Range Test*

*R. Ficaria* is the only host of the haplontic phase of the two strains of *U. rumicis*. Both in field experiments and in the climatic chamber aecia were produced only on this species.

In the climatic chamber the first pycnia were observed 2 wks after inoculation; three weeks later aecia with aeciospores appeared. In the field experiment aecia were observed in April. In every experiment a reinfection of *R. obtusifolius* with the aeciospores was successful.

No other plants showed any symptoms (necrotic or red spots) after inoculation, either in the climatic chamber or in the field experiment. Eleven species of the genus *Ranunculus*, 16 other Ranunculaceae species, 12 crop plants, *R. crispus*, *R. obtusifolius* and *Berberis vulgaris* L. (Berberidaceae) were tested (Tables 1 and 2). Some of these species were tested only in the climatic chamber (Tables 1 and 2).

A complete cycle from teliospores to teliospores (on detached dock leaves and *R. Ficaria* plants) required approximately 130 days.

#### **Discussion**

Teliospores of *U. rumicis* germinate in nature after overwintering (Gäumann 1931). With the method described in this paper it is possible to activate germination of teliospores under controlled conditions in a relatively short time (60 days). The method allows inoculation of test plants at a temperature convenient to the plants at the beginning of germination.

The activation of teliospore germination by storing teliospores on water agar agrees with results of Groth *et al.* (1978) and Gold and Mendgen (1983) for bean rust teliospores. The break of dormancy is probably due to aging and following rehydration of the spores.

The results of the host range presented in this paper confirmed the work of Tranzschel (1905, 1909) and Gäumann (1931). Tranzschel reported for the first time the connection between the rust *U. rumicis* on docks and an aecidium on *R. Ficaria*. Gäumann observed that aecia were induced only on *R. Ficaria* after a natural overwintering together with leaves bearing telia of *U. rumicis*. *R. Stevenii* auct. non Andr. (= *Friesianus* Jordan) and *R. bulbosus* L. were not infected. Attempts by Inman (1971) to induce pycnial and aecial infections by inoculations with teliospores were unsuccessful.

Frank (1973) suggests that *U. rumicis* overwinters, at least in the Rome area, mainly as uredia or vegetative mycelia on the primary host. Primary infection in spring is by uredospores, rather than aeciospores from the alternate host. This fact can increase the chance of successful biological control. The earlier in spring that uredospores appear, the better the possibility of control of docks.

Our studies have confirmed the high degree of specialization of *U. rumicis* in the haplontic phase. The lack of knowledge concerning the alternate host of the rust fungus was the only reason to prevent release of the organism for biological control of docks (Frank 1973). Our work has filled this gap and release is now possible.

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