Ramularia Rubella (Bon.) Nannf. as a potential mycoherbicide against Rumex weeds

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Ramularia Rubella (Bon.) Nannf. as a potential mycoherbicide against *Rumex* weeds

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Abstract

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The host range of the leaf fungus *Rumularia rubella* (Bon.) Nannf. is restricted to the subgenus *Rumex,* to which the most noxious weeds of the genus belong: *R. crispus* and *R. obtusifolius*. Repeated infections of *R. obtusifolius* L. with this pathogen stopped the growth of the weed in the greenhouse. Leaf number decreased and the dry matter of the roots was distinctly reduced: after 6 weeks it amounted to 59%, and after 11 weeks to 53% of that of non-inoculated control plants. In combination with other stress factors (other biocontrol agents, chill, drought, competition), it is possible that *R. obtusifolius* might thus be controlled in the field.

Introduction

Rumex obtusifolius L. is a major weed in pastures and greenlands of Central Europe. Together with *R. crispus* L. and *R. pulcher* L. it is known worldwide as a weed not only in these habitats but also in several crops (Neururer 1972, Holm et al. 1977, Anonymous 1983). Chemical control is expensive and not permanent: after damage the plants can soon put out new shoots from their tap root. Also, their seed stock in the soil is enormous: one plant can produce tens of thousands of seeds a year which remain viable for several decades (Cavers and Harper 1964).

For these reasons the above-mentioned weeds have been the target of investigations for biological control either with insects (Miyazaki and Naito 1974, Bentley and Whittaker 1979, Scott 1985, Scott 1988) or with fungal pathogens (Frank 1971, Inman 1971, Glasgow and Templeton 1983, Schubiger 1985, Strässle et al. 1986).

Ramularia rubella (Bon.) Nannf. (= *Ovularia obliqua* [Cooke] Oudem.) is a *Deuteromycete* (*Hyphomycetes*) which occurs endemically in Europe, the Americas and Asia. It causes red leaf spots about 1 cm in diameter on several *Rumex* spp. (Fig. 1).

The subject of this study was to investigate the potential of the fungus *Ramularia rubella* as a biological control agent against *Rumex* spp. The first step was to resolve the discrepancies in the literature concerning the host range of the fungus within the genus *Rumex* (Lindau 1907, Laibach 1921, Wollenweber 1932, Kerr 1961, Gunnerbeck 1967, Schubiger et al. 1983, Ellis and Ellis 1985, Strässle et al. 1986, Wilson 1986), the second was to investigate its effect on *R. obtusifolius* in the greenhouse.

Material and methods

Host range

The origins of the tested plants, collected as seeds, are shown in table 1. The seeds were sown in plastic pots (9.5 cm high, 11 cm in diameter) with a mixture of soil (Potgrond, de Baat, Netherlands), perlite and sand (granulation 1.5–2 mm) in the volume-ratio of 4:2:1. Five plants per pot of each origin were grown in the greenhouse. The temperature was held at 20 °C by day (16 h), and 15 °C by night; relative humidity of the air was about 80%. During the day, natural light was

Tab. 1. List of the *Rumex* spp. and their origins. The seeds were collected by G. Meinicke and W. Huber (Geobotanic Institute, ETH Zurich). * leg. M. Baltisberger, Geobotanic Institute, ETH Zurich. ** leg. W. Huber, Geobotanic Institute, ETH Zurich.

Species	Origin	Number
R. acetosa L.	Alesse VS; 1000 m. 570'500/110'900	6.1.3
R. acetosa L.	Pfäffikersee ZH; 540 m. 700'900/244'125	6.1.4
R. acetosella L.	Val de Galbe, Pyrénées Orientales, France; 1780–1900 m	6.2.5
R. acetosella L.	Locarno TI; 200 m. 704'700/112'700	6.2.6
R. alpinus L.	Region of the Vanil Noir FR; 1450 m. 581'000/154'850	6.22.10
R. alpinus L.	Road of the Gr. St. Bernhard VS; 1940 m. 581'175/83'075	6.22.11
R. alpinus L.	Road of the Julier GR; 1600 m. 768'175/154'060	6.22.12
R. aquaticus L	Schleitheim SH; 460 m. 676'700/289'400	6.3.3
R. arifolius All.	Grenchenberg SO; 1150 m. 569'950/230'550	6.23.2
R. arifolius All.	Pisciadel GR; 1430 m. 802'400/139'050	6.23.3
R. conglomeratus Murr.	Wunderklingen SH; 420 m. 673'220/284'450	6.6.4
R. conglomeratus Murr.	Tenero TI; 200 m. 709'500/114'300	6.6.6
R. crispus L.	Vigo di Fassa, Südtirol, Italy; 1400 m	6.7.23
R. crispus L.	Bözberg AG; 450 m. 649'900/258'750	6.7.24a
R. crispus L.	Wunderklingen SH; 420 m. 673'220/284'450	6.7.25
R. crispus L.*	Bremen, FRG	6.7.30
R. hydrolapathum Huds.	Wunderklingen SH; 415 m. 672'750/283'580	6.19.2
R. hydrolapathum Huds.	Bonfol JU; 440 m. 579'250/257'900	6.19.3
R. longifolius DC.*	Skive, 30 km NNE Holstebro, Denmark	6.10.2
R. longifolius DC.**	Szigliget, 15 km E Keszthely, Hungary; 150 m	6.10.3
R. maritimus L.	Bonfol JU; 440 m. 579'800/257'700	6.11.3
R. obtusifolius L.	Gebenstorf AG; 365 m. 660'250/257'700	6.12.15
R. obtusifolius L.**	Lenk BE; 1350 m. 601'650/144'400	6.12.17
R. obtusifolius L.	Wunderklingen SH; 420 m. 673'220/284'450	6.12.18
R. obtusifolius L.	Vigo di Fassa, Südtirol, Italy; 1400 m	6.12.20
R. patientia L.**	Kerecsend, 10 km S Eger, Hungary; 150 m	6.15.2
R. patientia L.*	Uludag, 20 km SE Bursa, Turkey; 1870 m	6.15.3
R. pulcher L.	Locarno TI; 200 m. 704'450/113'170	6.13.5
R. pulcher L.	Sondrio, Veltin, Italy; 350 m	6.13.6
R. sanguineus L.	Scherz AG; 430 m. 656'250/254'700	6.18.3
R. sanguineus L.	Wunderklingen SH; 420 m. 673'220/284'450	6.18.4
R. scutatus L.	Cavigliano TI; 320 m. 698'700/115'550	6.24.2
R. scutatus L.	Road of the Gr. St. Bernhard VS; 1940 m. 580'900/82'950	6.24.3

supplemented by artificial illumination (high-pressure metal-halide discharge lamps, Philips) for 16 h resulting in a light intensity of at least 23,000 lux. At the time of inoculation each plant had developed at least 3 leaves.

For inoculation a mixture of several strains of *R. rubella* was used (Tab. 2). These were propagated on carrot agar (250 ml carrot juice [Biotta]; 1.5 g $CaCO_3$; 20 g malt extract [Oxoid]; 20 g Bacto-Agar [Difco]; 750 ml tap water) in Petri dishes of 8.6 cm diameter. The conidia did not lose their pathogenicity during cultivation. Stock cultures were held on malt agar (2%).

The suspensions for inoculation were made by washing off the conidia from the Petri dishes with 0.05% of Etalfix (25% Citowett [isooctyl-phenyl ether of polyethylene glycol] diluted in 20% methyl alcohol; Maag AG, Switzerland). The suspensions were sprayed on the plants by pressurized air. Viable conidia were determined by germination tests on water agar (2%). After inoculation the plants were kept without artificial illumination at 100% relative humidity for 48 h.

The experiment was carried out twice (A and B). Control plants were kept in a separate chamber of the greenhouse.

Damage to R. obtusifolius

In preliminary tests the *R. rubella* strain G21b (Tab. 2) was selected from 12 strains collected from all over Switzerland as the most aggressive (it infected 75% of the leaf-surfaces of *R. obtusifolius* plants) (Meinicke 1987).

The plants were grown from seeds collected from *R. obtusifolius* (6.12.18, tab. 1). Seedlings with one leaf formed were transplanted into clay pots (18 cm high, 14.5 cm in diameter) filled with a mixture of half earth (low humic loam) and half sand (granulation 1.5–2 mm). They were watered with Knop's nutrient solution (1 g $Ca[NO_3]_2$; 0.25 g KH_2PO_4; 0.25 g $MgSO_4$; 0.125 g KCl; 10 mg Sequestren [Fe^{3+} chelated with ethylene-diamine-di-o-hydroxyphenylacetic acid, Ciba Geigy]; 1 ml A–Z-solution according to Hoagland [Ziegler 1978] in 1 litre H_2O).

The conditions in the greenhouse were the same as described above, but the temperature was maintained at 22 °C by day and 16 °C by night.

Thirty plants for treatment and 30 control plants were grown separately in two greenhouse chambers under the same conditions. At the beginning of the experiment the plants were 4 weeks old and had developed 4 to 6 leaves. The treatment plants were sprayed weekly with suspensions containing 10^5 to 10^6 conidia per ml (suspended in 0.05% Etalfix). Sixty to 140 ml of suspensions were used depending on the size of the plants. Germination tests were made on water agar (2%).

Tab. 2. Origin of the strains of *Ramularia rubella* and composition of spore suspensions used for the host range tests (experiments A and B).

Number	Host	Origin	Number of viable conidia (10^4/ml of suspension)	
			Experiment A	Experiment B
G8	*R. alpinus* L.	Weisstannental SG; 1360 m. 739'400/203'400	11	7
G21b	*R conglomeratus* Murr.	Wunderklingen SH; 420 m. 673'220/284'450	23	15
G12	*R. crispus* L. × *R. obtusifolius* L.	Bözberg AG; 450 m. 649'900/258'750	1	0.1
G13	*R. obtusifolius* L.	Boalp ZH; 1000 m. 715'350/239'750	2	4
G19	*R. obtusifolius* L.	Lenk BE; 1350 m. 601'650/144'400		2
G15	*R. sanguineus* L.	Scherz AG; 430 m. 656'250/254'700	11	7

After inoculation all the plants (control plants included) were kept without artificial illumination at 100% relative humidity for 48 h. After 6 weeks of infection half of the plants (15 control and 15 treatment) were harvested, the other half after 11 weeks of infection.

At harvest the roots of each plant were washed and the number of assimilating leaves was counted. The dry matter of roots and leaves of each plant were determined separately after drying for 24 h at 105 °C. The whole experiment was carried out twice (A and B). The two experiments were analysed separately.

Results

Host range

The differences in the amounts of viable conidia in experiments A and B were too small to cause differences in the degree of infection (Tab. 2, 3).

The host range of *R. rubella* was restricted to the subgenus *Rumex* (tab. 3). Within this taxon all species became infected: *R. aquaticus* L., *R. hydrolapathum* L. and *R. patientia* L. were attacked only weakly; *R. alpinus* L., *R. conglomeratus* Murr., *R. crispus* L., *R. longifolius* DC., *R. maritimus* L., *R. obtusifolius* L., *R. pulcher* L. and *R. sanguineus* L. were heavily infected. Species outside the subgenus *Rumex* were not infected.

Tab. 3. Host range of *Ramularia rubella*. A mixture of conidia of the strains G8, G21b, G12, G13, G19 and G15 was used as inoculum (Tab. 2).

Subgenus	Plants[a]	Infection after 14 days	
		Experiment A	Experiment B
Rumex	*R. alpinus* L.		
	6.22.10	++	++
	6.22.11	++	++
	6.22.12	++	++
	R. aquaticus L.		
	6.3.3	+	++
	R. conglomeratus Murr.		
	6.6.4	++	++
	6.6.6	++	++
	R. crispus L.		
	6.7.23	++	++
	6.7.25	++	++
	6.7.30	++	++
	R. hydrolapathum Huds.		
	6.19.2[b]	+	
	6.19.3[c]	+	++
	R. longifolius DC.		
	6.10.2	++	++
	6.10.3	++	++
	R. maritimus L.		
	6.11.3	++	++

Tab. 3. (continued).

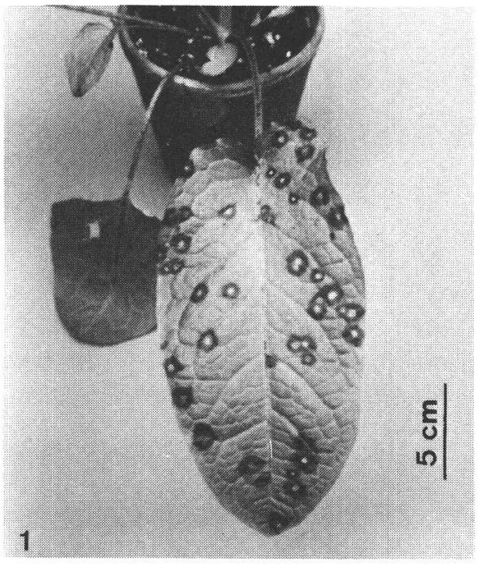
Subgenus	Plants[a]	Infection after 14 days	
		Experiment A	Experiment B
	R. obtusifolius L.		
	6.12.15	+ +	+ +
	6.12.17	+ +	+ +
	6.12.18	+ +	+ +
	6.12.20	+ +	+ +
	R. patientia L.		
	6.15.2	+	+ +
	6.15.3	+	+
	R. pulcher L.		
	6.13.5	+ +	+ +
	6.13.6	+ +	+ +
	R. sanguineus L.		
	6.18.3	+ +	+ +
	6.18.4	+ +	+ +
Acetosa	*R. acetosa* L.		
	6.1.3	–	–
	6.1.4	–	–
	R. arifolius All.		
	6.23.2	–	–
	6.23.3	–	–
	R. scutatus L.		
	6.24.2	–	–
	6.24.3	–	–
Acetosella	*R. acetosella* L.		
	6.2.5	–	–
	6.2.6	–	–

–: No leaf spots
+: 1–10 leaf spots per plant
+ +: > 10 leaf spots on several leaves
[a] 5 plants/origin (Tab. 1)
[b] In experiment A 1 plant, in experiment B no plant
[c] In experiment A 2 plants, in experiment B 5 plants

Damage to R. obtusifolius

About 90% of the conidia germinated during each inoculation. First symptoms in the form of red spots appeared after 3–5 d. The fungus sporulated on the young leaves after 7 d, on the older ones after 10 d.

Only the severely infected leaves (more than 50% of the leaf area infected) died within 7 to 10 d after inoculation. Less infected leaves survived but their assimilating area was reduced. The infected plants lost many leaves but they also produced more new leaves than the control plants. For this reason the total number of leaves was not always significantly reduced (Tab. 4).



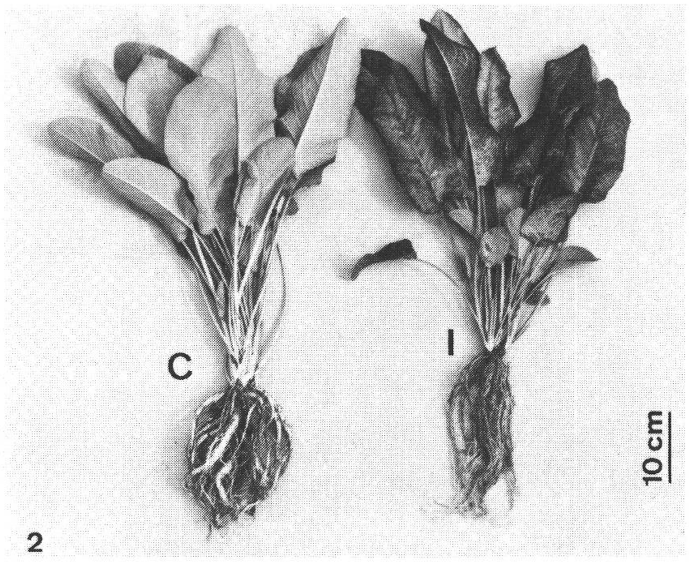


Fig. 1. Leaf spots of *Ramularia rubella* on *Rumex obtusifolius*.

Fig. 2. Damage to *Rumex obtusifolius* after 11 weeks of infection with *Ramularia rubella* (Experiment B). C: non-inoculated control plant I: infected plant.

Tab. 4. Effect of weekly inoculations of *Ramularia rubella* (strain G21b, Tab. 2) on the number of leaves, and on the dry matter of leaves and roots of *Rumex obtusifolius* (6.12.18, Tab. 1) (A, B: 2 independent experiments; statistical differences between control and infection according to Student's t-test: * $P \leq 0.01$, ** $P \leq 0.001$, $^{n.s.}$ not significant).

		Harvest after 6 weeks		Harvest after 11 weeks	
		Control	Infection	Control	Infection
Number of assimilating leaves per plant	A	34.2 ± 7.4	28.5 ± 11.8 $^{n.s.}$	70.2 ± 8.0	40.1 ± 16.4**
	B	26.7 ± 10.5	13.9 ± 7.7**	49.6 ± 16.5	42.6 ± 16.5 $^{n.s.}$
Dry matter of leaves (g/plant)	A	12.6 ± 3.4	10.4 ± 2.7 $^{n.s.}$	44.1 ± 11.5	18.3 ± 4.5**
	B	9.5 ± 3.1	5.6 ± 1.6**	27.5 ± 12.9	22.3 ± 5.1 $^{n.s.}$
Dry matter of roots (g/plant)	A	9.3 ± 2.9	5.5 ± 3.4*	37.4 ± 15.8	20.5 ± 9.3**
	B	6.2 ± 3.5	3.6 ± 1.2*	36.1 ± 14.8	18.8 ± 9.5**

During experiment A, the plants began to form additional rosettes at the time of the first inoculation. Therefore, they were able to produce more new leaves and the number of leaves at the first harvest was scarcely reduced. At the second harvest, however, the number of leaves was reduced to 57% of that of the control plants.

During experiment B, additional rosettes were formed later so that at the first harvest there was a great reduction (to 52%) in the number of leaves, whereas at the second harvest no significant reduction could be observed.

The dry matter of the leaves was not reduced; it was approximately proportional to the number of leaves.

The extra leaves of the infected plants, however, obviously derived energy from the root stock: the dry matter of the rhizome was significantly reduced to 59% (experiment A) and 58% (experiment B) already at the first harvest; at the second harvest a reduction to 55% and 52% respectively (Tab. 4) was observed. The extension of the fine roots as well as the root stocks were reduced (Fig. 2).

Discussion

Host range

Earlier host-range tests including many crop plants besides some *Rumex* spp. showed that *R. rubella* infected none of the crop plants tested (Schubiger et al. 1983, Strässle et al. 1986). Moreover, according to most of the literature citations only species of the subgenus *Rumex* become infected (Lindau 1907, Laibach 1921, Wollenweber 1932, Kerr 1961, Gunnerbeck 1967, Schubiger et al. 1983, Strässle et al. 1986). Ellis and Ellis (1985) and Wilson (1986), however, also named *R. acetosa* L. as a host. For these reasons we tested all species of the subgenus *Rumex* occurring in Switzerland except *R. cristatus* L., which is known from one place only (Hess et al. 1967). Three of 4 species of the subgenus *Acetosa* (*R. acetosa* L., *R. arifolius* All., *R. scutatus* L.) and one of three species of the subgenus *Acetosella* (*R. acetosella* L.) were also included in our tests. They did not become infected. The species within these two subgenera are closely related, which is shown by frequent hybridization and geneintrogression (Hess et al. 1967, den Nijs et al. 1985). It can thus be supposed that the non-tested species of these subgenera can also not be infected by *R. rubella*.

Of the infected species (all within the subgenus *Rumex*) only *R. aquaticus* L., *R. maritimus* L. and *R. sanguineus* L. are unknown as weeds (Anonymous 1983). The former two occur only rarely in Switzerland but they would not be threatened by application of *R. rubella* as a mycoherbicide, because their habitats (wet places), as well as those of *R. sanguineus* (damp and shady places), are isolated from those of the weeds.

The most noxious weed *R. crispus* and *R. obtusifolius* (Holm et al. 1977) were heavily attacked.

Damage to R. obtusifolius

Repeated inoculations were required because the fungus could not spread by itself in the greenhouse in the absence of wind and rain. Under natural conditions in the field one application of conidia may be sufficient to establish the fungus in a weed-population, and wind and rain could then further disseminate the fungal spores.

Removing the cauline leaves of *R. crispus* caused a severe reduction of the larger seeds (Maun and Cavers 1971). The remaining smaller seeds exhibited lower dormancy and produced less vigorous seedlings (Maun and Cavers 1971). The same effect may be expected with *R. obtusifolius*. Like defoliation, infection of leaves with *R. rubella* (observed on cauline leaves as well as on leaves of the rosettes) reduced the assimilating area. As *R. obtusifolius* seedlings have poor competitive ability (Cavers and Harper 1964), this further decrease could render the establishment of *R. obtusifolius* from seeds more difficult.

Other findings show that if infected *R. obtusifolius* plants are exposed to other stress factors, increased damage can be expected. For example, experiments of Inman (1971) with the rust fungus *Uromyces rumicis* (Schum.) Wint. showed that only 43% of rusted *R. crispus* survived the winter compared with 95% of non-rusted control plants. Schubiger et al. (1986) showed that this rust fungus reduced the dry matter of both leaves and roots of *R. crispus* to 35%. *R. rubella*-infected *R. obtusifolius* is likely to show a similar reaction.

High-intensity grazing of the chrysomelid beetle *Gastrophysa viridula* Degeer on *R. obtusifolius* resulted in a reduction of root dry matter to 63%, whereas the leaf dry matter was reduced to 18% (Bentley and Whittaker 1979). *R. obtusifolius* was only weakly checked by sole competition with *R. crispus*, whereas a combination of competition and grazing with the beetle resulted in increased weight losses (Bentley and Whittaker 1979).

Increase of *R. obtusifolius* is strictly correlated with availability of nutrients (Jeangros 1985): if it was grown in an established population of *Lolium perenne* L., its development was reduced apparently because of root competition for nitrogen. Reduction of photosynthetically active radiation also reduced the vigour of the plants drastically (Jeangros 1985). In a population of *Poa pratensis* L. with low nitrogen supply (fertilisation of 120 kg N/ha), shaded *R. obtusifolius* almost failed to regrow after cutting (Niggli 1985).

The greatest problem with *R. obtusifolius* arises from the rhizome. The seedlings accumulate carbohydrates in the roots from the third week on (Jeangros 1985). This nutritional stock permits the plant to regrow quickly after damage to the leaves. Adult plants regenerated their carbohydrate reserves in the roots three weeks after cutting the leaves (Hidaka 1973); frequent cutting reduced the content significantly.

In our greenhouse experiments, the plants had a surplus nitrogen supply. Earlier infection of the seedlings as well as persistent infection of adult plants, combined with root interactions with other plants should result in increased damage to the weed. Whether or not *R. rubella* infection together with natural stress factors such as drought, chill, competition for light and nutrients could lead to a control of *R. obtusifolius*, should now be tested in the field.

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