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Ecological Characteristics of Introduced Species, *Rumex acetosella*

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도입종 애기수영의 생태 특성

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ABSTRACT

Ecological characteristics of *Rumex acetosella* have been studied both in the laboratory and in the field. *R. acetosella* grows well straight up after germination, elongating and thickening their rhizomes with sprouts erupting along their bodies of the rhizome. The number and development of leaves and ramets reach their peak by April. The size of leaves and the cover degree of the plant increase from April to May. *R. acetosella* is an ephemeral, a short life mode plant, finishing its life cycle in July or August.

The germination and seedling growth of selected species exposed to aqueous extracts of *R. acetosella* were in inverse proportion to concentration. The growth of *Rumex japonicus* and *Digitaria sanguinalis* cultivated in soil with *R. acetosella* was more inhibited than that of the control.

Twelve chemical compounds were identified from *R. acetosella* by GC and HPLC and verified by bioassay with the same chemical reagents. It would be assumed that these chemical substances are responsible for the allelopathic effect of *R. acetosella*. In short, *R. acetosella* plants grow vigorously from April and maintain their superior competitive ability to other plants in forming their communities by emitting chemical substances into their environment.

Key words: *Rumex acetosells*, Growth characteristics, Ephemeral, Aqueous extracts, GC and HPLC, Allelopathic effect.

INTRODUCTION

Rumex acetosella cannot grow near by *Rumex crispus* (Einhellig and Rusmussen 1973). *Rumex acetosa* can't grow with *Poa pratensis*, *Poa annua* or *Trifolium dubium* (Turkington *et al.* 1985). *P. pratensis* can't mingle and survive near a place with *Rumex*

acetosella (Fowler and Antonovics 1981).

The plants emit their own secondary metabolites into their enviroment to suppress other plants seed germination or seedling growth even though they stunt their own growth or kill themselves (Hull and Muller 1977). There are different kinds of these natural toxic chemicals. Among them, phenolic compounds and terpenoids are the most powerful (Grummer 1961, Chou and Chen 1976).

R. acetosella is a perennial and belongs to the Polygonaceae and is an introduced species from Europe. This plant is distributed everywhere in South Korea as a naturalized plant and occupies roadsides or old fields of farming and mountain areas. So, it causes a lot of damage to other plants because it forms communities dominantly. Moreover, this species grows thickly and spreads widely. The consequence of this grouping ability is that *R. acetosella* plants dominate and sometimes totally exclude other species in the same habitat.

The purpose of the present study is to determine *Rumex acetosella's* growth characteristics in a hillside area through field survey and laboratory work. We have investigated *R. acetosella's* life cycle and found several factors related to their adaptative ability.

MATERIALS AND METHODS

We have studied *R. acetosella's* growth in both lab experiments and field surveys. Field surveys were performed on hillsides of Yonho-ri, Ongdong-myon, Chong-up-gun and Mt. Miruk of Iksan city by quadrat method (50 cm × 50 cm). The growth of *R. acetosella* was monitored by photographs from April, 1996 to March, 1997. And *R. acetosella* plants were brought to the lab then planted in pots and measured every two days.

The growth characteristics

We monitored the growth of *R. acetosella* monthly both in the field and laboratory by modified Choung's method (1989). Length (L) and width (W) of the leaves chosen from sampled *R. acetosella* plants were measured, then the rates of growth were calculated (W/L). The leaves were put to photosensitive paper and fixed with ammonia water to measure growth of leaves.

We set up 15 quadrats (50cm×50cm) at their habitat and counted the number of fresh and dead leaves for given periods and the number of new ramets monthly. The cover percent of *R. acetosella* plants in the quadrat was measured, and after we selected plants, then investigated shoot growth, root growth, photosynthetic and nonphotosynthetic parts of the plant.

Experimental materials

R. acetosella was chosen as donor plants. Recipient species were *Raphanus sativus* var. *hortensis* for. *acanthiformis*, *Malva verticillata*, *Oenothera odorata*, *Chrysanthemum coronarium* var. *spationsum*, *Gnaphalium affine*, *Poa nemoralis*, *Rumex japonicus*, *Digitaria sanguinalis*, *Allium sativum* for. *pekinense* cv. Nangimaneul, and *Oryza sativa* cv. Dongjinbyeo.

Germination and growth

The method used to prepare aqueous extracts of *R. acetosella* was by Kil *et al.* (1989). Fifty seed grains of the recipient species were selected, seeded on Petri dishes (d. 12cm) with two sheets of filter paper and wetted with 10ml of extract for germination. The extracts were given to them every two days with an equal amount of distilled water provided as the control. Seedling length was measured 7days after sowing.

We put soil into pots (d. 14cm) and did growth experiments by the same method as above. These experiments were done 4 times and all data were used for statistical analysis.

Tissue culture

The method of tissue culture followed Kil *et al.*

(1993).

Analysis of chemical substances

We took 2 g of the *R. acetosella* plant and removed oils with n-hexane, mixed with 70 % methanol and 70% acetic acid (1:1), added this to the mixed-liquid and separated it centrifugally, filtered it and concentrated it, then separated centrifugally again with 6M HCl which was adjusted to pH 2.0. We extracted this 5 times with n-hexane : DW (1:1), made a TMS inducement and injected it into a gas chromatograph (GC).

We added 400ml methanol to a 400g soil sample and treated it to supersonic waves for 30 minutes, then filtered it. We analyzed it by GC after being decompressed, concentrated, and dried with 7-hydroxycoumarin 200 mg as an ISTD and made a TMS inducement. Every component from standard compounds made TMS inducement and was analyzed by GC and identified by comparison of retention times. The column was an SE-54 (30cm × 0.25mm) fused silica capillary, column temperature 90℃ (5 minutes) increased to 250℃ (20 min.) at 4℃/min., FID injector was 280℃, gas flow was N_2 1.87ml/min. and split ratio was 36.1.

RESULTS AND DISCUSSION

The growth pattern of *Rumex acetosella*

R. acetosella ramets were recruited by two different ways. One was by seed propagation that received a new ontogeny by seed germination. The other was by vegetative propagation by sprouting from rhizomes. *R. acetosella* plants grew straight up for a year after their germination (A and B from Fig. 1). And they sprouted successively from the long rhizome in the second year. Then new shoots were formed and grew up branching continuously (Fig. 1).

According to Crawely (1990), *R. acetosella* was propagated by asexual reproduction from the vegetat-

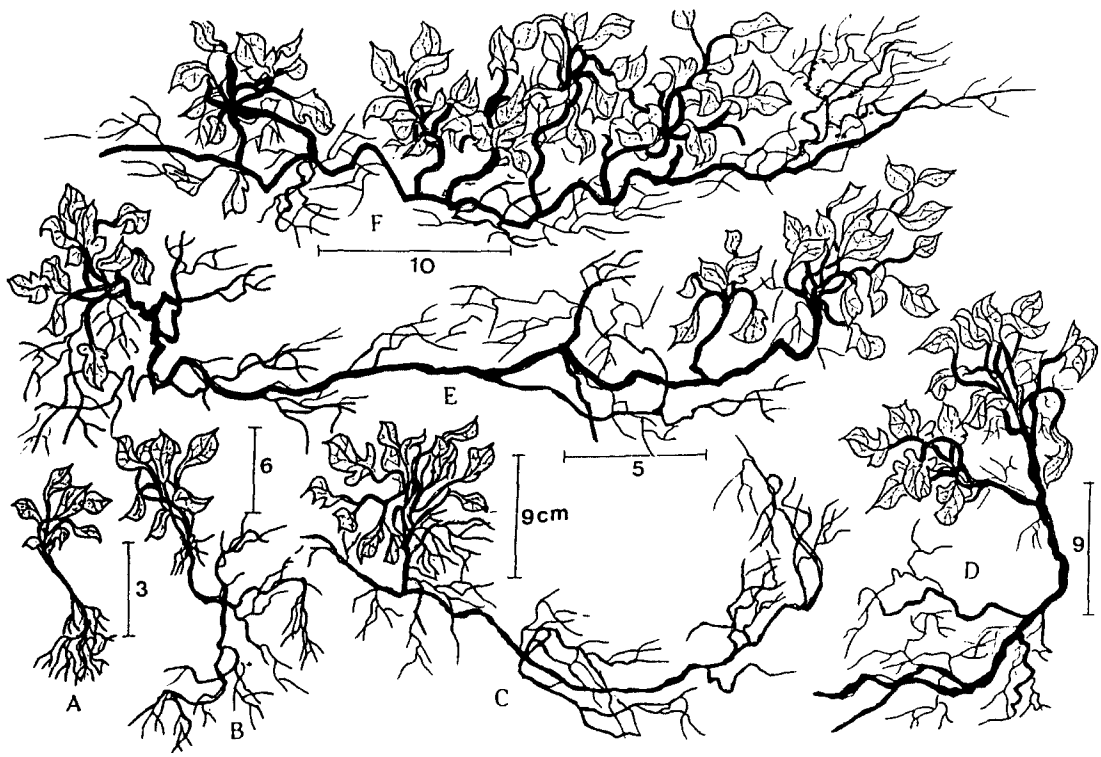

Fig. 1. The growth morphology of *Rumex acetosella*.

ive organ rather than by seed propagation. That was the reason why some *R. acetosella* damaged by grazing were able to survive. Vezina and Bouchard (1989) indicated that *R. acetosella* plants produce a creeping stems between July and October, to compete with other plants. These creepers do lots of harm to competitor plants the following spring. For example, the creepers make strawberry yields decrease as much as 50%, and delay ripening. If the strawberry plants were furnished with water, injurious effects from *R. acetosella* were increased. The length, the width and the leaf size of *R. acetosella* were measured between April and July during the vigorous growing. As a result, the length increased vigorously from May and recorded maximum in June, but decreased in July. The growth rate of the length differed significantly by 5% every month. In the case of leaf width, they did not change remarkably. But the growth of the width in May was decreased from April and then it increased again between June and July. The size of the leaves grew from April and grew to the maximum in July. After that, they began to wilt and die (Table 1).

After 20 leaves of *R. acetosella* were chosen, the length and width were measured and calculated the W/L value is shown in Fig. 2.

This was showed that the growth of the leaves was increased actively between May and June. We researched the number of fresh and dead leaves in the fi-

Table 1. Monthly changes of leaf growth of *Rumex acetosella* grown in the field

Month	Leaf length(cm)* Mean ± S.D	Leaf width(cm)* Mean ± S.D	Leaf area(cm^2)* Mean ± S.D
April	1.762 ± 0.700^{b}	0.620 ± 0.094^{AB}	1.299 ± 384.593^{B}
May	2.927 ± 0.698^{ab}	0.501 ± 0.105^{A}	1.476 ± 407.798^{B}
June	3.362 ± 1.022^{a}	0.610 ± 0.152^{AB}	1.699 ± 555.641^{B}
July	2.700 ± 0.533^{ab}	0.793 ± 0.096^{A}	3.199 ± 333.417^{A}

*Means followed by the same letter with a column are not significantly different according to Duncan's multiple-range test. a, ab : $p < 0.05$, A, B show $p < 0.01$.

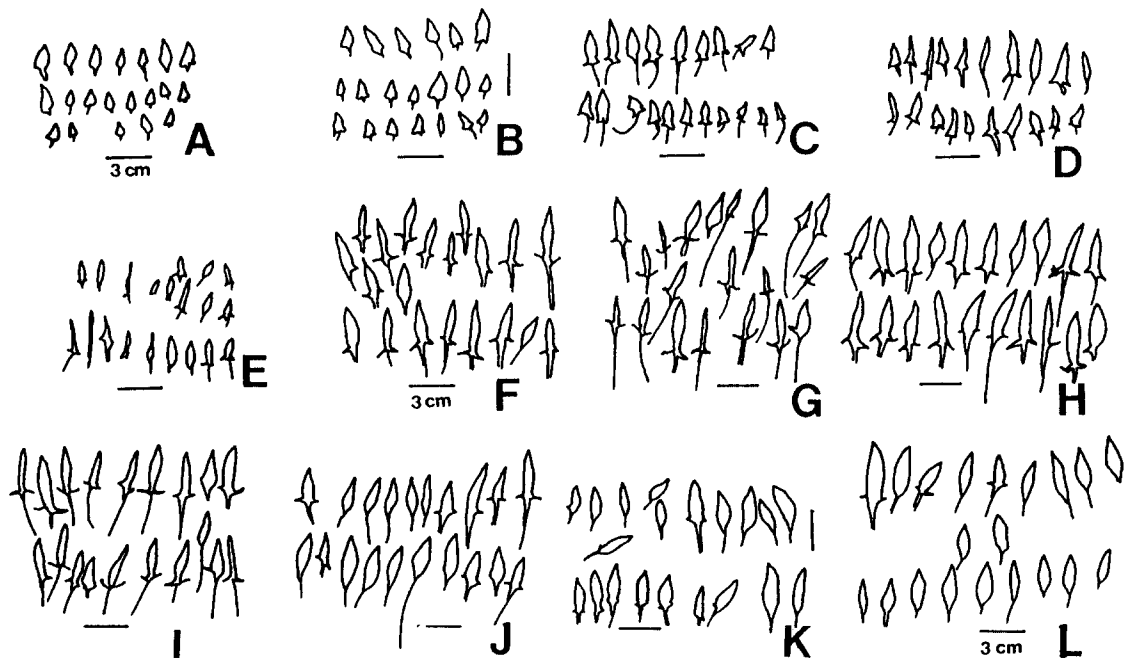

Fig. 2. Monthly change of leaf length(L), leaf width/leaf length(W/L) of *Rumex acetosella*. The leaf morphology, in general, is changed from elliptical and heart-shaped leaf in April to hastate and/or ablong included different shapes in June or July. In photographs bar are corresponded 3 cm and the mean data of W/L are measured as follows: A(W/L 0.48, April 6th), B(0.28, April 20th), C(0.47, April 27th), D(0.21, May 4th), E(0.23, May 11th), F (0.18, May 19th), G(0.56, May 25th), H(0.17, June 1st), I(0.18, June 8th), J(0.21, June 22nd), K(0.31, July 6th), L (0.41, July 20th).

eld habitat (50cm×50cm). The results follow in Fig. 3.

The number of fresh leaves decreased from April but that of dead leaves increased. It seemed that *R. asetosella* almost complete their life cycle between July and August.

Monthly development of ramet was shown in Fig. 4.

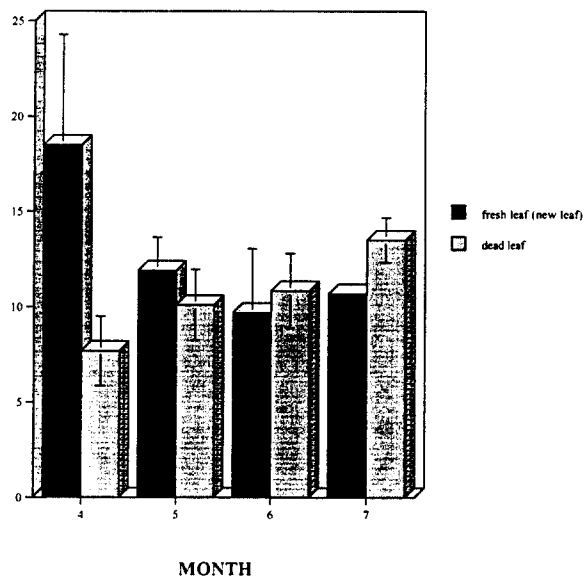

Fig. 3. Monthly variation in the number of fresh and dead leaves of *Rumex acetosella*.

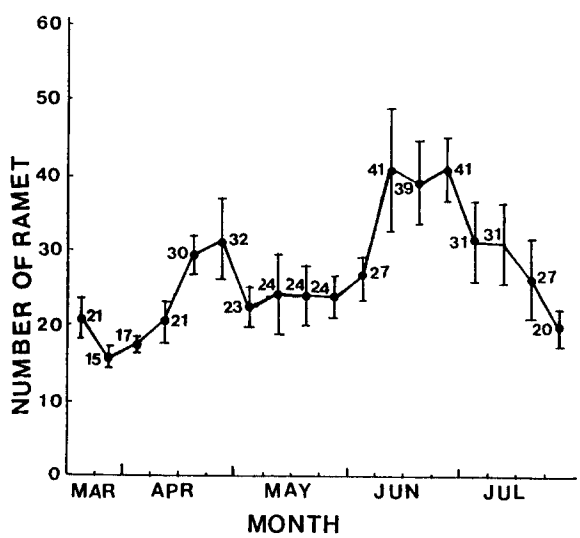

Fig. 4. Changes in the number of ramets of *Rumex acetosella* in sampled sites at 4~7 days intervals from March to July. Vertical bars are SD and numbers indicates mean caculated.

Ramets increased as the same trend with increasing rate of fresh leaves in Fig. 3. This suggested that ramets increased rapidly in April and decreased from May gradually.

Cover degree of *R. acetosella* in the sampling sites (50cm × 50cm) kept increasing from April to Mayand stayed the same till July (Fig. 5).

In Canada, *R. acetosella* is the main weed comprising of all wild weeds. In other words, *R. acetosella* occupied about 80% frequency of whole weeds (Thomas and Ivany 1990). Changes of coverage demonstrated that *R. acetosella* was relatively grown over time and showned to go up and down through the growing season. It seemed that because of vigorous growth of *R. acetosella*, this species might be occupied dominantly in field partly.

Shoot and root growth of *R. acetosella* showed in Fig. 6.

Shoots and roots kept increasing from April to July. The result of fresh weight of *R. acetosella* in the field area (50cm×50cm) is in Table 2. Photosynthetic parts increased between March and April, increased much in May and then decreased in July. Nonphotosynthetic parts also increased between March and April and both parts decreased a little in July

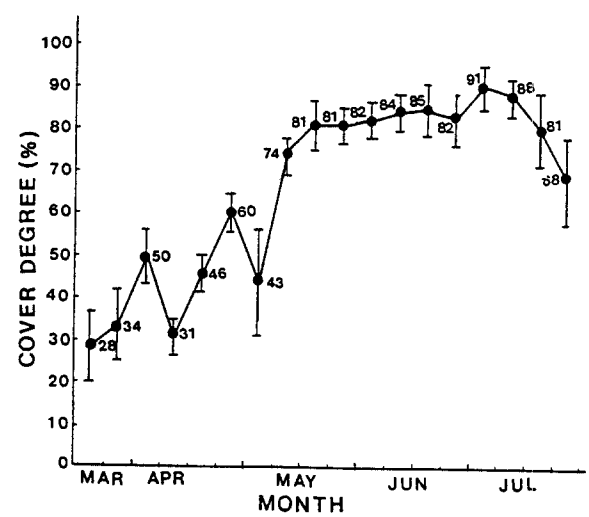

Fig. 5. Changes of cover degree of *R. acetosella* in sampled sites at 4~7 days intervals from March to July. Vertical bars are SD and number indicates mean calculated.

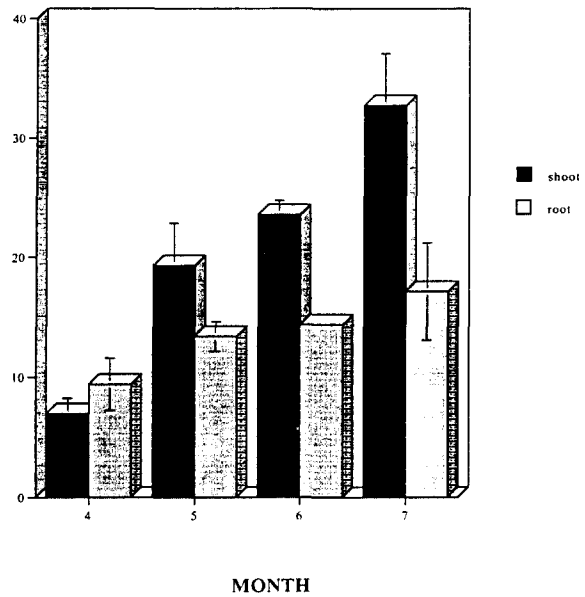

Fig. 6. Comparison between shoot and root length (mm) of *Rumex acetosella*.

Table 2. Fresh and dry weight of *Rumex acetosella* grown for 5 months in field. Initial biomass for young plant of *R. acetosella* did not determine

Manth	Photosynthetic part	Non-photosynthetic part
	Mean ± S.D.	Mean ± S.D.
	Fresh weight	
March	3.510 ± 0.651	19.850 ± 0.071
April	8.485 ± 3.228*	49.450 ± 2.520*
May	13.925 ± 2.226**	52.245 ± 6.413*
June	13.170 ± 0.597**	60.833 ± 3.570**
July	12.637 ± 3.246**	59.910 ± 3.118**
	Dry weight	
March	5.196 ± 0.602	14.505 ± 2.553
April	6.764 ± 0.826*	26.498 ± 12.134
May	7.027 ± 0.203**	23.997 ± 3.831
June	6.600 ± 1.091*	27.897 ± 5.057
July	7.400 ± 0.437**	30.717 ± 3.329*

Values were means ± SD. An asterisk indicated that the differences between monthly growth for that species were statistically significant($p < 0.05$, $p < 0.01$).

(Table 2).

The photosynthetic parts changed monthly, but there were no differences in nonphotosynthetic parts. Also the number of flower was 3.5 on April 22nd, 10 on April 30th, 15 on May 7th, 18 on May 15th, 21 on May 22nd, 23 on May 29th, 26 on June 4th, 4th, 31 on June 11st, 36 on June 19th, 28 on June 25th, 20 on July 2nd and 10 on July 9th. In a short day photoperiod the *R. acetosella* plant was inthe shape of a rosette, but turning to long day conditions it showed flowers at about 130~140 days (Bavrina *et al.* 1991).

Germination and seedling growth with extracts

Germination and growth of 4 species treated with aqueous extracts of *R. acetosella* were tested and the

Table 3. Seed germination and seedling elongation(mm) of four species sown in petri dish treated with aqueous extracts of *Rumex acetosella*

Species	Control	Extraction time(hr)				
		24	48	72	96	120
	Seed germination					
Raphanus sativus var. *hortensis* for. *acanthiformis*	40.0^{a}	29.3^{b}	39.8^{a}	39.8^{a}	30.0^{b}	14.8^{c}
Malva verticillata	44.0^{a}	31.5^{c}	41.0^{ab}	34.8^{bc}	19.3^{d}	12.5^{e}
Oenothera odorata	47.8^{a}	41.8^{b}	48.8^{a}	48.5^{a}	38.8^{b}	37.0^{b}
Chrysanthemum coronarium var. *spatiosum*	17.5^{a}	15.3^{a}	16.0^{a}	13.0^{a}	4.3^{b}	0.8^{b}
	Seedling elongation					
Raphanus sativus var. *hortensis* for. *acanthiformis*	115.4^{a}	60.8^{bc}	67.7^{b}	54.7^{c}	12.2^{d}	10.5^{d}
Malva verticillata	63.1^{a}	47.3^{b}	58.1^{a}	47.0^{b}	12.6^{d}	21.2^{c}
Oenothera odorata	22.1^{a}	15.6^{b}	11.2^{c}	12.5^{c}	8.0^{d}	8.1^{d}
Chrysanthemum coronarium var. *spatiosum*	23.1^{a}	14.3^{abc}	16.0^{ab}	13.0^{abc}	4.5^{c}	7.2^{bc}

*Means within a row followed by same letters are not significantly different at the 5% level by Duncan's multiple-range test.

results were shown in Table 3.

The germination rate of the test plot was lower than that of the control. But in the test plot of *Oenothera odorata* the germination rate was higher than the control at 48 and 72hrs. In general, except for the first period of 24hrs, the longer the extraction time took, the lower the germination rate showed. The germination rate of the test plot against the control was 58.5~9.1%, 92.1~19.9, 70.6~36.2 and 69.3~19.5 in *Raphanus sativus* var. *hortensis* for. *acanthiformis*, *Malva verticillata*, *O. odorata*, and *Chrysanthemum coronarium* var. *spationsum*, respectively. If the extracting time was longer, the growth of the seedlings was more clearly inhibited.

In soil underneath *R. acetosella* and soil without the plants, shoot and root growth of *Gnaphalium affine* and *Poa nemoralis* showed almost no differences from the control (Table 4).

But that of *Rumex japonicus* and *Digitaria sanguinalis* was retarded compared to that of control. This suggested that *G. affine* and *P. nemoralis* plants which occurred in similar habitats with *R. acetosella* species were less affected by residual chemical substances from *R. acetosella's* soil. In the matter of residual toxic substances of habitat soil, this study is similar to the experiment for the relationship between the pine tree and other plants (Kil and Yim 1983).

Selected plant growth test with *R. acetosella* stand soil (test) and control soil, growth was suppressed at test soil. Datta and Sinha-Roy (1974) pointed out chemical substances from plants can linger even as weathering, leaching, exudating, diffusion, decomposition and volatilization take place. The present experiment seemed to relate with Shindo and Kuwatsuka (1976) that the chemical substances from the plant were left in the soil and continue to inhibit the growth of other plants.

Tissue culture

Aqueous extract of *R. acetosella* added to MS 121 media and used for callus induction of garlic plants. As the extract concentration from *R. acetosella* increased, garlic callus induction decreased. Even though the callus was induced well, it was recorded that

Table 5. Callus induction of *Allium sativum* for. *pekinense* cv. Nangimaneul and *Oryza sativa* cv. Dongjinbyeo on MS121 medium *Rumex acetosella* extracts after 30 days culture

Extract concentration (%)	Callus induction (%)		
	++	+	−
Allium sativum for. *pekinense* cv. Nangimaneul			
0 (Control)	100	0	0
10	62.5	30	7.5
20	62.5	15	22.5
40	52.5	9.5	38.0
60	35.0	5.5	59.5
Oryza sativa cv. Dongjinbyeo			
0 (Control)	90.0	4.0	6.0
10	77.5	20.0	2.5
20	62.5	15.5	22.0
40	65.0	7.0	28.0
60	47.5	2.5	50.5

++, good ; +, bad ; −, none

Table 4. Seedling growth (cm) of experimental species tested in control (abandoned field soil without *Rumex acetosella*) and test soil (with *R. acetosella*)

Species	Shoot elongation (cm)		Root elongation (cm)	
	Control	Test	Control	Test
	Mean ± S.D.			
Gnaphalium affine	17.14 ± 1.72	16.80 ± 3.09	9.25 ± 1.80	8.57 ± 2.99
Poa nemoralis	2.95 ± 0.21	2.97 ± 2.31	1.39 ± 1.25	1.33 ± 0.60
Rumex japonicus	4.94 ± 1.38	3.60 ± 1.27	4.04 ± 1.62	1.77 ± 1.43**
Digitaria sanguinalis	10.10 ± 2.09	6.56 ± 0.87**	6.56 ± 0.38	5.41 ± 1.12

**, $p < 0.01$

there was 62.5% induction in 10% and 20% extracts. But low rate (35%) was shown in 60% extract of *R. acetosella* (Table 5).

This is similar to the result of callus induction of rice plants. It was proved that aqueous extracts from *R. acetosella* had a toxic effect on callus induction of garlic and rice plants.

Analysis of chemical components

To identify the natural chemical substances in *R. acetosella*, HPLC and GC were used and the results follow in Table 6.

The components of leaves, roots and soils from *R. acetosella* were identified as being citric acid, malonic acid and succinic acid. The main compound in leaves and root was oxalic acid. Six kinds of phenolic acid were also identified by GC. The secondary metabolites which affect the basic growth of the plant were phenols, terpenoids, flavonoids and alkaloids. The phenolic compounds were related to the control of germination and growth of the plants (Kapustka and Rice 1976, Rasmussen and Einhellig 1979). Also these facts have been proved by Carballeira (1980), Shettel and Balke (1983) and Shindo and Kuwastsuka (1976). They have reported that phenolic compounds including ferulic acid were verified as the primary inhibitor against germination and seedling growth. The present experiment also identified, by GC, six phenolic compounds such as caffeic acid from the *R. acetosella* plant. Other researchers found many commom compounds similar to our study from *Pinus densiflora* needles by GC (Kil and Yim 1983), from *P. thunbergii* needle by PC and HPLC and from *Artemisia princeps* var. *orientalis* leaves by GC (Kil 1989). Bioassy was done with identical compound chemical reagents detected from the *R. acetosella* plant and these chemical substances had an inhibition effect more upon dry weight growth than on seed germination. That is, in the 5×10^{-5}M concentration of phenolic compounds, the germination rate of the control was 67% but dry weight rate was 51% in a lettuce experiment. These results are similar to the 16 different kinds of plants from an abandoned field (Stowe *et al.* 1987).

Table 6. Chemical substances identified from *Rumex acetosella* by HPLC and GC

Chemical substances	Leaf	Root	Shoot	Soil
HPLC				
Oxalic acid	52.0	36.9		–
Malonic acid	5.56	7.26		0.012
Fumaric acid	1.34	0.91		–
Succinic acid	2.45	1.36		0.15
Malic acid	22.9	18.4		–
Citric acid	23.1	43.1		0.33
GC				
Caffeic acid		0.40	0.40	
Protocatechuic acid			0.46	
Ferulic acid			0.62	
Vanillic acid			0.74	
Pyrogallol		0.82		
Gentisic acid		0.86		

적 요

애기수영 식물의 생태 특성을 구명하기 위하여 실험실과 야외조사를 병행 실시하였다. 애기수영 식물은 발아 후 직립 위주의 신장생장을 하고 뒤이어서 지하경을 뻗어 옆으로 불어나며 그 위에 새로운 개체가 돋아나고 더 많은 분지와 새 개체가 증가하는 왕성한 생장을 하였다. 잎과 ramet의 수는 4월에 최고치를 기록했고 잎 면적과 식물의 피도는 4월부터 5월까지 계속 증가하였다. 애기수영은 그 후 7월부터 8월에 생활환을 마치는 단명식물로 판명되었다.

애기수영 식물의 수용성 추출액에 대한 발아율과 신장율은 실험식물별로 다르지만 대체로 추출액의 농도에 반비례적인 경향을 나타냈다. 애기수영 식물이 나있던 임상토양에 식물을 재배실험한 결과 참소리쟁이와 바랭이의 유식물 생장은 비교적 억제적이었다.

한편 애기수영 식물을 GC와 HPLC로 분석하여 12종류의 화학물질을 분리했고 그 중 이들과 동일한 몇 종류의 시약으로 생물학적 정량을 실시한 결과로 보아 이들이 애기수영의 allelopathy 작용에 관계가 깊은 물질의 일부일 것으로 추정되었다.

그래서 애기수영 식물은 4월 경부터 왕성한 생장으로 주위의 다른 식물보다 경쟁력 우세를 보이는 군락을 형성하며, 아울러 화학물질을 주위환경에 방출하여 다른 식물보다 우위를 차지하는 생태 특성이 있다는 사실을 밝혀냈다.

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