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Rusts for the biological control of leafy spurge (*Euphorbia esula*) in North America

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Systemic teliospore infections of 4 rusts (*Uromyces* spp.) have been collected in Europe from plants morphologically similar to the target North American weeds. The systemic nature of the infections and the apparently rapid kill of infected plants early in the growing season emphasize the potential value of these rusts in a biological control programme. However, this potential has yet to be realized since the conditions necessary for teliospore germination of perhaps the most promising species *Uromyces scutellatus,* remain unresolved. For 3 other species, *U. laevis*, *U. euphorbiae* and an unidentified species, the basidiospores produced following teliospore germination gave rise to short internal mycelium within inoculated Canadian spurge, but further development was restricted by callose formation by the host. Urediniospore stages of *Melampsora euphorbiae* have also been collected although germination has been inconsistent, and where germ tubes and appressoria were formed there was no penetration of the inoculated target plants. Failure to promote infection has prompted chemotaxonomic studies into the leafy spurge complex in order to clarify the host:rust interrelationships. Gas chromatographic analysis of the triterpenoid contents of latex has shown this to be a stable characteristic that can be interpreted as being typical for individual species. A close relationship between European and North American spurges, as indicated by similar profiles, has enabled an assessment of those areas in Europe in which spurge plants might most successfully be surveyed for the collection of biological control agents compatible with North American weedy species.

Introduction

In Europe the leafy spurges *Euphorbia esula* L. and *E. waldsteinii* Sojac (Euphorbiaceae) occur only in relatively limited stands, and are not problem weeds, yet in North America leafy spurges have spread extensively since their introduction from Europe, to cover wide areas of rangeland (Dunn 1979). Chemical control is uneconomic and largely ineffective. Biological control offers a viable alternative and with the introduction of host-specific flea beetles (*Apthona* spp.; Coleoptera: Chrysomelidae) from Europe there has been a reduction in the spurge populations of drier areas (P. Harris, personal communication). Rusts of spurges such as *Melampsora euphorbiae* (Schub.) Cast., and *Uromyces* spp. (Uredinales) have potential as biological control agents since they appear to be common and highly damaging diseases in Europe. The systemic nature of the *Uromyces* spp. in particular would favour their use as biological control agents of a plant, such as spurge, that spreads vigourously by rhizomes. Yet, despite the apparent success of the rusts in their native range their potential as biological control agents remains unfulfilled, in part as a consequence of incompatibility between agents collected from European plants when challenged against the North American target species. The introduced North American spurges have unclear affinities with their Eurasian counterparts despite numerous studies into the taxonomic position and phylogenetic relationships of plants in the subsection *Esula* (Prokh.). Traditional taxonomic studies describe numerous taxa for leafy spurge (Radcliffe-Smith 1985), in contrast Crompton *et al.* (1990) and Stahevitch *et al.* (1988) presented evidence that leafy spurge should be considered as a single, polymorphic species (*E. esula*).

In this paper we assess progress towards the use of rusts for the biological control of leafy spurge, and consider the relationship between European spurges and adventive Canadian plants. Following Crompton *et al*. (1990) here we consider leafy spurge to be *E. esula*; however, it is worth noting that according to Radcliffe-Smith (1985), our European leafy spurge accessions included *E. esula*, *E. waldsteinii*, *E. pseudovirgata* and *E. pseudoesula.*

Spurge rusts in Europe

The telial infections of the *Uromyces* spp. collected on European leafy spurges to date have been systemic in nature with hyphae growing through the vascular tissue of infected plants. Rusted shoots are considerably weaker than uninfected stems and generally do not flower or branch. Although telia are usually formed on every leaflet of an infected shoot adjacent shoots produced from the same rhizome are not necessarily infected.

Of the rusts associated with the European spurges, *U. laevis* Koern. on *E. segueriana* Neck., and an as yet unidentified *Uromyces* species *(W912)* (indet. nr. *U. sublevis* Tranzsh.) on *E. nicaeensis* All. were 2 apparently specific species. *U. scutellatus* (Pers.) Lev. has a wider host range having been collected from *E. esula*, *E. waldsteinii*, *E. cyparissias* L., and *E. lucida* (Waldst. & Kit.), however the host range appeared to be restricted to members of the Series Esula. This species together with *U. kalmusii* Sacc. collected from *E. esula* in the Caucasus, may have the most potential as biological control agents although the infection biology of, and conditions necessary for, teliospore germination have yet to be resolved. *U.*

euphorbiae Unam. was perhaps of less interest as it occurred in North America as well as Europe on a number of *Euphorbia* spp., but not *E. esula.* In addition to these species, symptoms of which occur relatively early in the growing season, another rust infection, caused *by Melampsora euphorbiae*, can become a prominent disease on *E. esula*, *E. waldsteinii*, *E. salicifolia* Host., and *E. cyparissias* in late June. Bruckart *et al.* (1986) considered that specific races of this rust could have a sufficiently restricted host range to be considered for biological control if isolates pathogenic to North American leafy spurge could be collected. They concluded that a greater understanding of the evolution of North American spurges and their relationship to European forms was required to explain their observations of host incompatibility and enhance the prospects for collection of suitable races.

Infection studies

Teliospores of *U. laevis*, *U. euphorbiae* and *W912* germinated to produce viable basidiospores after 2-5 d high RH at 17-22°C. However the basidiospores did not infect Canadian leafy spurge, and in most cases appressoria were formed without subsequent penetration. Occasionally the rust did penetrate the host but was restricted from further development by the deposition of callose by the host around the point of infection.

The conditions necessary for the germination of *U. scutellatus* teliospores appeared to be more exacting. Defago *et al.* (1985) considered that infection by *U. scutellatus* occurred through the rhizome bud, with symptoms not appearing until 18 months after infection had occurred.

In our studies teliospores inoculated onto rhizome buds have not germinated, even following the inoculation of rhizome from the original host plant. The influence of host plant materials, temperature, period of inoculation (from 24 hours to 30 days), and storage conditions for spores up to 3 years old have been examined, but none of the variations tested has induced teliospore germination. Teliospore germination can be stimulated in certain other rust fungi by plant extracts (Binder *et al.* 1977, Van Den Ende *et al.* 1987). Such a stimulus may be required for germination of *U. scutellatus* teliospores, as a mechanism to prevent reduction of inoculum potential in the absence of susceptible host plant biotypes.

Germination of urediniospores of *Melampsora euphorbiae* has been inconsistent. Germ tubes and appressoria have been produced but further development with penetration of Canadian target plants has not occurred, implying that the conditions for infection have been achieved but not with the correct host biotype.

Chemotaxonomy of leafy spurge

Ebke and McCarty (1983) and Harvey *et al.* (1988) demonstrated the use of morphological characteristics alone to be of only limited value in separating the leafy spurges. Mahlberg *et al.* (1987) demonstrated that the latex triterpenoid content of members of the Euphorbiaceae could be interpreted by gas chromatography to be stable and characteristic for individual species. The use of gas chromatography to assess latex triterpenoid profiles of the European and North American accessions could assist in their

identification and elucidate the European origins of North American adventive plants, closely related plants possibly sharing similar profiles. By matching profile types we hoped to be able to assess those areas in Europe in which spurge plants might most successfully be surveyed for the collection of biological control agents compatible with North American target plants.

Latex was collected into acetone-washed vials, stored and subsequently extracted and analysed according to the procedures of Mahlberg *et al.* (1987). Latex samples were taken from rusted and non-infected leafy spurge plants. Collections were made from across Europe to allow an assessment of the extent of variation in profile forms for individual species. In addition the degree of variation within geographically distinct populations was examined in samples collected from 2 particular sites in the Pest and Hadju-Biharn regions of Hungary where leafy spurge formed the dominant vegetation. The sites were chosen because of their relatively large size for European stands, the apparent morphological uniformity of the population and the occurrence of numerous rusted plants of either *M. euphorbiae* or *U. scutellatus.* Both sites have been locations for the collection of phytophagous insects compatible with Canadian and North American leafy spurge. In total 173 accessions of *E. esula* were analysed, with 54 accessions taken from the Pest site and 47 accessions from the Hadju-Biharn site.

Analysis of latex from European spurges other than *E. esula* including *E. lucida* Waldst. & Kit., *E. cyparissias* L., *E. segueriana* Necker, *E. nicaeensis* All., *E. pallustris* L., and *E. epithymoides* L. (Table 1), confirmed that the latex profile was diagnostic for each

Table 1. Source and number of accessions collected in Europe.

Taxon	Origin of Collection		Number of Accessions
	Country	Location	
Euphorbia esula	Hungary:	Hadju-Beharn (3 sites)	61
		Pest (2 sites)	59
		Csongra	10
		Szoinok	10
	Yugoslavia:	Serbia (3 sites)	17
	Eastern Austria:	(3 sites)	12
	Southern Czechoslovakia:	(2 sites)	4
E. cyparissias	Hungary:	Pest (1 site)	8
		Bacs-Kiskun (3 sites)	16
	Eastern Austria:	(2 sites)	3
E. lucida	Hungary:	Bacs-Kiskun (1 site)	4
	Yugoslavia:	Serbia (1 site)	4
E. segueriana	Hungary:	Bacs-Kiskun (3 sites)	8
	Germany:	(1 site)	2
E. steopposa	Hungary:	Veszprem (2 sites)	6
E. pallustris	Hungary:	Hadju-Biharn (1 site)	3
	Yugoslavia:	Serbia (1 site)	6
E. epithymoides	Yugoslavia:	Serbia (1 site)	2

Table 2. Percentage composition of triterpenoid profiles of European leafy spurge accessions.

Taxon	Peak Number and Retention Time (min)[1]									
	1 10.2	**3 10.9**	**6 13.2**	**7 14.0**	**8 14.3**	**10 15.0**	**11 15.2**	**12 15.8**	**13 16.9**	**13a 17.6**
Euphorbia sequeriana	–[2]	3 ±0.5	–[2]	12 ±1.0	10 ±1.3	7 ±2.1	9 ±0.5	37 ±3.2	22 ±1.8	–[2]
E. cyparissias	–[2]	–[2]		17[3] ±6.8	–[2]	11[3] ±0.8	–[2]	33 ±3.8	39 ±7.7	–[2]
E. lucida	–[2]	–[2]		14[3] ±4.4	–[2]	4 ±0.5	–[2]	±2.2	71[3] ±2.6	–[2]
E. nicaeensis	–[2]	–[2]	20 ±1.5	–[2]		18[3] ±1.7	32 ±3.7	30 ±4.8	–[2]	–[2]
E. epithymoides	1 ±0.6	–[2]	–[2]	–[2]	–[2]	–[2] –[2]	6 ±1.4	4 ±1.6	89[3] ±7.1	–[2]
E. pallustris	–[2]	–[2]	–[2]	–[2]	–[2]	–[2]	5 ±0.7	10 ±1.2	66 +3.2	19 ±1.1

[1]Relative retention time corrected to internal standard of 6.43 min for all chromatograms.
[2]Not detected.
[3]Shoulder associated with peak-indicates position relative to peak.

individual taxon (Table 2). However profiles from *E. esula* were much more variable and could be separated into groups on the basis of qualitative and quantitative differences in triterpenoid composition. Analysis of rusted and non-rusted shoots from the same rhizome indicated that the triterpenoid profile was not modified by apparent infection. Similar profile types were grouped with each group assigned a letter for identification. Grouped profiles were compared to the profiles from samples obtained from 40 Canadian accessions comprising 8 collections from each of 5 sites in Alberta (Beverley Bridge), Saskatchewan (Mortlach, Maxim), and Manitoba (Brandon, Spruce Woods park).

Twenty profile types were identified from the 173 European *E. esula* accessions, a few of which are presented here. It appeared that the extent of variation in profile composition of the European populations was greater than from Canadian populations as a whole and also when compared for individual sites, possibly indicating that Canadian populations were established from a relatively small number of introductions. Certain European *E. esula* collections produced profiles that included peaks not observed in Canadian profiles. Peak 6 (RT 10.5) was identified in a number of European collections represented in 6 groups, including profile groups L and J (Table 3). Significantly this peak was present in all profiles from plants that, according to Radcliffe-Smith (1985) would be ascribed as *E. esula.* Peak 9 (RT 14.7) was present in profile form F, and peak 17 (RT 19.5) was represented in 2 European groupings H and I.

The presence of these unique features formed the basis for our separation of the collections although the taxonomic significance remains unresolved.

Table 3. Percentage composition of triterpenoid profiles of European leafy spurge accessions.

Group	Peak Number and Retention Time (min)[1]									
	2 **10.5**	**7** **14.0**	**8** **14.3**	**9** **14.7**	**11** **15.2**	**12** **15.8**	**13** **16.9**	**14** **17.8**	**15** **18.9**	**17** **19.5**
A	–[2]	2[3] +1.1	–[2]	–[2]	5[3] ±1.0	27 ±5.4	55 ±5.5	1.2 ±3.5	–[2]	–[2]
B	–[2]	5[3] ±0.3	–[2]	–[2]	5[3] ±1.2	25 ±4.1	55 ±6.3	10 ±2.2	–[2]	–[2]
E	–[2]	3[3] ±0.7	–[2]	–[2]	7[3] ±0.6	26 ±3.8	64 ±3.1	tr[4]	–[2]	–[2]
F	–[2]	21 ±0.4	–[2]	5 ±0.6	6 ±0.1	1.4 ±0.8	34 ±1.1	20 ±2.0	–[2]	–[2]
G	–[2]	–[2]	–[2]	–[2]	8 ±0.2	43 ±1.1	37 ±1.3	12 ±0.2	–[2]	–[2]
H	–[2]	1 ±0.2	–[2]	–[2]	6 ±0.6	19 ±0.5	59 ±1.1	12 ±0.8	2 +1.4	1 ±0.1
I	–[2]	1 ±0.2	–[2]	–[2]	2 ±1.0	17 ±3.3	6.1 ±6.4	17 ±0.5	–[2]	2 ±0.6
J	2 ±0.4	3 ±0.8	1 ±0.1	–[2]	6[3] ±2.7	45 ±5.5	36 ±7.0	7 ±0.8	–[2]	–[2]
L	8 ±3.0	7 ±1.6	7 ±1.1	–[2]	14[3] +3.5	26 ±2.5	38 ±4.4	–[2]	–[2]	–[2]

[1]Relative retention time corrected to internal standard of 6.43 min for all chromatograms.
[2]Not detected.
[3]Shoulder associated with peak-indicates position relative to peak.
[4]Peaks comprising <0.5% total triterpenoid content.

Despite the apparent variability of chemotypes, identical profiles were detected among accessions collected from different sites within Europe, and perhaps more significantly between collections from Europe and Canada.

The group A profile type was identified from three European sites in the Csongrad, Pest and Hadju-Biharn regions, and also from 2 Canadian sites (Beverley Bridge and Mortlach) (Table 4). This profile represented the most common form collected from the Pest site and consequently future rust collections from there will be screened particularly against plants from these target populations. Type B, also identified from accessions collected at the Pest site, matched profiles of accessions from Mortlach and Brandon, and profile type E present in 2 European populations in the Csongrad and Pest regions matched profiles from Beverley Bridge. However, none of the profiles from plants collected at Spruce Woods Park or the Maxim location matched any of the European forms.

Table 4. Percentage composition of triterpenoid profiles of Canadian leafy spurge accessions.

	Peak Number and Retention Time (min)[1]					
Group	**7 14.0**	**8 14.3**	**11 15.2**	**12 15.8**	**13 16.9**	**14 17.8**
A	3[3] ±1.1	–[2]	5 ±1.0	22 ±2.2	60 ±7.8	10 ±3.5
B	5[3] ±0.7	–[2]	4[3] ±0.8	20 ±3.4	57 ±3.6	14 ±3.1
E	3[3] ±0.6	–[2]	6[3] ±1.3	22 ±1.0	69 ±2.8	tr[4] –[2]

[1]Relative retention time corrected to internal standard of 6.43 min for all chromatograms.
[2]Not detected.
[3]Shoulder associated with peak-indicates position relative to peak.

Conclusion

The wide range of triterpenoid profile types produced by both Canadian and European accessions could not have been predicted merely from morphological characteristics. The results do not resolve whether leafy spurge should be considered as a single variable species consisting of a number of biotypes or chemotypes, or rather as a complex of a number of species, since the taxonomic significance of the profile composition has yet to be resolved. However, despite this the implications for biological control necessitate making collections from plants in Europe that most closely resemble particular North American types and targeting releases of biological control agents to areas in North America with an infestation of a comparable plant type.

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