

***Puccinia distincta* AND *Puccinia lagenophorae*, TWO RUST FUNGI OF ASTERACEAE RECENTLY INTRODUCED INTO SLOVENIA**

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ABSTRACT

The current distribution of two rust fungi on Asteraceae, *Puccinia distincta* McAlpine on wild daisies (*Bellis perennis* L.) and *Puccinia lagenophorae* Cooke on groundsel (*Senecio vulgaris* L.), is described for Slovenia. The diagnostic features of both rust species are described using Slovenian material. Field observations suggest that *P. lagenophorae* is well-established across Slovenia whereas *P. distincta* is a recent arrival still spreading across the country. Although both species have an opsis-type life-cycle with aeciospores as the main carrier of infection and also share many morphological features, their different epidemiologies support their classification as two distinct species.

Key words: *Bellis perennis*, *Puccinia distincta*, *Puccinia lagenophorae*, *Puccinia obscura*, *Senecio vulgaris*, Slovenia

IZVLEČEK

RJI NA NEBINOVKAH, *Puccinia distincta* IN *Puccinia lagenophorae*, OD NEDAVNEGA V SLOVENIJI

Prikazana je sedanja razširjenost dveh rj na nebinovkah (*Asteraceae*) v Sloveniji, *Puccinia distincta* McAlpine na marjeticah (*Bellis perennis* L.) in *Puccinia lagenophorae* Cooke na navadnem grintu (*Senecio vulgaris* L.). Opisane so diagnostične značilnosti obeh vrst rj na vzorcih iz Slovenije. Terenska opazovanja nakazujejo, da je *P. lagenophorae* že dobro ustaljena po vsej Sloveniji, *P. distincta* pa je nedavni prišlek, ki se še širi po deželi. Čeprav imata obe vrsti opsis-obliko življenjskega kroga z eciosporami kot nosilci okužb in imata mnoge morfološke značilnosti enake, pa njuna različna epifitotologija podpira njuno uvrstitev v dve različni vrsti.

Ključne besede: *Bellis perennis*, *Puccinia distincta*, *Puccinia lagenophorae*, *Puccinia obscura*, *Senecio vulgaris*, Slovenija

1. INTRODUCTION

Two recent epidemics of rust fungi on members of the Asteraceae have attracted particular attention from European plant pathologists who have been able to witness their rapid spread across the entire continent. These epidemics are caused by *Puccinia*

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lagenophorae Cooke mainly on groundsel (*Senecio vulgaris* L.) but also on other *Senecio* spp., and by *P. distincta* McAlpine on wild and cultivated daisies, *Bellis perennis* L. Both these rusts are autoecious and have opsis-type life-cycles with aeciospores as the main carrier of infection. Teliospores are occasionally formed and are capable of producing basidiospores upon germination, but these seem unable to re-infect the respective host plants (Weber *et al.*, 1998b). The similarities between these two rusts have led some authorities to consider them to belong to the same species, *P. lagenophorae* (Scholler, 1997; Gullino *et al.*, 1999). Others have distinguished the two rusts on the basis of teliospore morphology as well as lack of cross-infection of daisies by *P. lagenophorae* and of *Senecio* by *P. distincta* under experimental conditions (Weber *et al.*, 1998 a, b; Müller, 2000).

The course of the *P. lagenophorae* epidemic on *Senecio* has been well-documented (Viennot-Bourgin, 1964; Wilson *et al.*, 1965; Gjúrum, 1994; Scholler, 1994, 1997; Müller, 1995). The rust is probably of Australian origin and was first noticed in France in 1960 and in Britain in 1961. It was then discovered in Switzerland in 1962, Ireland in 1963, Tunisia in 1964, Germany in 1966, Greece in 1969, the Canary Islands in 1970, Romania in 1974, Austria in 1975, Yugoslavia (Croatia, Istria) in 1984 and Norway in 1993. However, the present paper appears to be the first record of its occurrence in Slovenia. Infections by *P. lagenophorae* and by subsequent secondary pathogens are so debilitating to the host that attempts are being made to employ this rust fungus as a biological control agent against *S. vulgaris* which can cause significant economic losses as a weed (Hallett & Ayres, 1992; Frantzen & Hatcher, 1997).

Puccinia distincta also seems to be of Australian origin and was first noticed there about 100 years ago when it caused such devastation of ornamental daisies that their cultivation had to be given up locally (McAlpine, 1906). The disease is similarly dramatic in Europe at present, although cultivated forms of *B. perennis* can still be grown if protected by fungicide sprays (Weber & Tilston, 1999; Gerlach, 2000). Despite the striking disease symptoms, the course especially of the early stage of the *P. distincta* epidemic on *B. perennis* is enigmatic, possibly due to collectors having confused it with *P. lagenophorae* or mis-identified it as *P. obscura* J. Schröter, a macrocyclic rust alternating between *B. perennis* and *Luzula* spp. (Jurc & Weber, 2000). Thus, although the daisy rust epidemic was not described until recently (Scholler, 1997; Weber *et al.*, 1998a), there is anecdotal evidence that serious and typical *P. distincta* infections on ornamental but not wild daisies may have occurred in Britain as early as 1981 (Preece *et al.*, 2000). The current pan-European epidemic probably arose in about 1995 due to the development of a new strain with an enhanced capacity to infect the ubiquitous wild daisies (Preece *et al.*, 2000). Here we report the presence of *P. lagenophorae* on groundsel and of *P. distincta* on wild daisies in Slovenia, and we describe the key features of identification for both rusts.

2. MATERIALS AND METHODS

Populations of wild *Bellis perennis* plants growing in lawns or cultivated forms in flowerbeds as well as *Senecio vulgaris* growing as a weed in parks and gardens were examined from July 2000 onwards at several sites across Slovenia. The disease incidence was recorded as the proportion of infected plants and scored as trace (<10% of plants diseased), light (11-30%), moderate (31-50%), or heavy (>50%). Details of the sampling sites, together with their Flora Europaea quadrant, are given in the Results section. Infected plants were investigated for macroscopic symptoms *in situ*, and representative herbarium material has been deposited at the Herbarium of the Slovenian Forestry Institute.

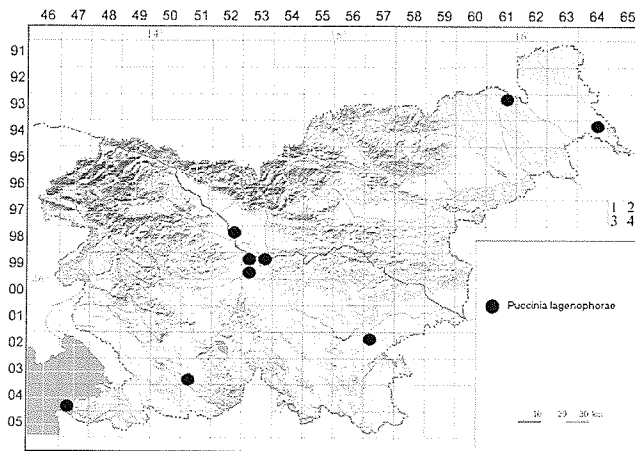
The dimensions of spores were measured to 0.5 μ m accuracy using dried material mounted in distilled water. For each sample, 25 such measurements were made of aeciospores and two-celled teliospores, and 10 measurements of one- or three-celled teliospores. In addition, the stalks at their point of attachment to the spore were measured for all teliospore types.

3. RESULTS

Infections of *S. vulgaris* were found in all parts of Slovenia as indicated in ure 1. Detailed records of infections are as follows: 1. Knežak (quadrant 03 51/3), aecia and telia present, heavy infection (6 Aug.). Wild *B. perennis* plants in the vicinity were free from infection. 2. Šentjakob near Ljubljana (99 53/2), aecia and telia, moderate (16 Aug.). Wild *B. perennis* free from infection. 3. Novo Mesto (02 57/1), aecia, light (14 Aug.). 4. Ljubljana Botanical Gardens (99 53/3), aecia and telia, light (19 Sept.). Wild *B. perennis* free from infection. 5. Vodice (98 52/2), aecia, light (9 Oct.). Wild *B. perennis* free from infection. 6. Ljubljana-Moste (99 53/1), aecia, light (25 Oct.). Wild *B. perennis* free from infection. 7. Ljubljana-Bežigrad, Agricultural Institute of Slovenia (99 53/1), aecia, light (27 Oct.). Wild *B. perennis* also infected. 8. Gornja Radgona (93 61/2), aecia, trace (28 Dec.). Wild and ornamental *B. perennis* free from infection. 9. Lendava (94 64/2), aecia, trace (28 Dec.). Wild and ornamental *B. perennis* free from infection. 10. Piran, cemetery (04 47/3), aecia, trace (15 Jan. 2001). Wild and ornamental *B. perennis* free from infection. Light aecial infections were also seen in Serbia (Belgrade, 30 Oct.) and in Croatia (two locations along the Belgrade-Zagreb motorway, 28 Oct.). In both cases, wild *B. perennis* populations in the immediate vicinity were free from infection.

Figure 1: Records of *Puccinia lagenophorae* on *Senecio vulgaris* in Slovenia (6 August 2000 - 15 January 2001).

Slika 1: Najdbe glive *Puccinia lagenophorae* na *Senecio vulgaris* v Sloveniji (6. avgust 2000 - 15. januar 2001).

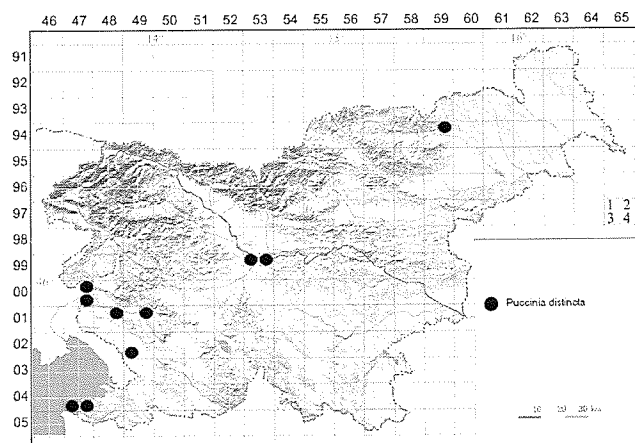


In contrast to infections of *S. vulgaris*, the distribution of rusted *B. perennis* plants was focused mainly on western Slovenia (ure 2). Infections were found at the following sites: 1. Portorož (04 47/3), aecia and telia, heavy (12 July). 2. Lucija (04 47/4), aecia and

telia, light (12 July). 3. Ljubljana-Polje (99 53/2), aecia and telia, heavy but localised (13 July). 4. Sežana Botanical Gardens (02 49/3), aecia and telia, heavy (8 Aug.). 5. Strunjan (04 47/4), aecia and telia, moderate (9 Aug.). 6. Branik (01 48/2), aecia and telia, moderate but localised (9 Aug.). 7. Nova Gorica (00 47/2), aecia and telia, moderate but localised (9 Aug.). 8. Vipava (01 49/2), aecia and telia, light (9 Aug.). 9. Maribor City Park (94 59/2), aecia and telia, generally trace but locally heavy (16 Aug.). 10. Ljubljana-Bežigrad, cinema (99 53/1), aecia, heavy but localised (4 Sept.). 11. Ljubljana-Bežigrad, Agricultural Institute of Slovenia (99 53/1), aecia, heavy but localised (27 Sept.). *S. vulgaris* also infected. 12. Miren (00 47/4) aecia, moderate (3 Oct.). Except for sample 11, *S. vulgaris* was not found in the vicinity of infected *B. perennis*.

Figure 2: Records of *Puccinia distincta* on wild *Bellis perennis* in Slovenia (12 July - 3 Oct. 2000).

Slika 2: Najdbe glive *Puccinia distincta* na negojenih *Bellis perennis* v Sloveniji (12. julij - 3. oktober 2000).



Wild *B. perennis* free from infection were seen at numerous places throughout Slovenia, including Bled, Kranj, Ilirska Bistrica, Kočevje, the motorway Celje-Maribor, and Ptuj. Extensive host populations entirely free from the disease were also seen on Pokljuka and the Mežakla Plateau at an altitude of 1200-1600 m. Infections of cultivated daisies were not seen by the authors, but reliable reports were received from Ljubljana and Nova Gorica (dr. Franci Celar and mag. Gabrijel Seljak, personal communication).

Aecia of *P. lagenophorae* were circular in outline, with a strongly protruding peridium (Figure 3B), and were abundant on stems as well as leaves of *S. vulgaris* (Figure 3A). Heavily infected plants were characterised by contorted stems and wilting leaves, leading to the onset of premature senescence. Telia, in contrast, were elongated and were found only on stems of the host, often occurring in clusters (Figure 3B). Aecia of *P. distincta* (Fig. 4B) resembled those of *P. lagenophorae* and were found on the leaves, petioles and inflorescences of wild *B. perennis*. Infected leaves had an uneven surface, often with inrolled margins, and were in a more upright position than the healthy,

rosette-type leaves (Fig. 4A). Heavy infections caused chlorosis and necrosis, especially in dry and warm weather. The irregularly-shaped telia were found on leaves (Figure 4B) and, more frequently, at the base of inflorescences (Figure 4C) and the abaxial midrib of petioles.

Microscopically, *P. lagenophorae* and *P. distincta* were very similar at first glance. Aeciospores of both species were spherical to subspherical in outline, often slightly angular, with regions of distinct but minute roughening alternating with smooth areas (Figures 3C, 4D). They measured $11.0\text{-}18.0 \times 10.5\text{-}15.0 \mu\text{m}$ (*P. lagenophorae*) or $12.0\text{-}20.0 \times 10.5\text{-}16.5 \mu\text{m}$ (*P. distincta*) and contained numerous small orange-coloured lipid droplets. Further, whilst both rust species produced two-celled teliospores and a certain proportion (about 10%) of one-celled spores (mesospores), three-celled teliospores were found only in *P. lagenophorae* (Figures 3C, 4D). The two rust species also differed markedly in the appearance of the teliospore stalk which was significantly wider at its point of attachment to the spore body in *P. lagenophorae* (Table 1) than in *P. distincta* (Table 2).

Table 1: Spore measurements (average \pm standard deviation) of *Puccinia lagenophorae* Cooke from wild *Senecio vulgaris* L. collected from Slovenia.

Preglednica 1: Velikost trosov (poprečje \pm standardni odklon) glive *Puccinia lagenophorae* Cooke iz *Senecio vulgaris* L. nabranih v Sloveniji.

	Sample 1 (Knežak)	Sample 2 (Šentjakob)	Sample 4 (Ljubljana)
Aeciospores (n = 251)	15.1 \pm 1.1 \times 12.9 \pm 0.9	14.7 \pm 1.1 \times 12.3 \pm 1.0	14.2 \pm 1.2 \times 12.2 \pm 1.1
1-celled teliospore body (n = 10)	25.8 \pm 3.8 \times 15.5 \pm 2.8	29.2 \pm 4.0 \times 15.1 \pm 1.2	25.2 \pm 2.7 \times 14.9 \pm 1.9
1-celled teliospore stalk (n = 10)	7.2 \pm 1.5	7.1 \pm 1.3	7.2 \pm 0.7
2-celled teliospore body (n = 25)	45.9 \pm 4.7 \times 20.1 \pm 1.8	43.8 \pm 4.9 \times 19.9 \pm 1.9	41.4 \pm 4.2 \times 18.7 \pm 1.5
2-celled teliospore stalk (n = 25)	8.1 \pm 1.3	8.6 \pm 1.0	8.6 \pm 0.9
3-celled teliospore body (n = 10)	53.5 \pm 5.7 \times 20.2 \pm 1.8	50.5 \pm 4.3 \times 21.5 \pm 2.1	43.4 \pm 3.7 \times 20.6 \pm 2.5
3-celled teliospore stalk (n = 10)	7.1 \pm 1.1	8.3 \pm 1.6	8.0 \pm 1.1

Table 2: Spore measurements (average \pm standard deviation) of *Puccinia distincta* McAlpine from wild *Bellis perennis* L. collected from Slovenia.

Preglednica 1: Velikost trosov (poprečje \pm standardni odklon) glive *Puccinia distincta* McAlpine iz negojenih *Bellis perennis* L. nabranih v Sloveniji.

	Sample 3 (Polje)	Sample 4 (Sežana)	Sample 9 (Maribor)
Aeciospores (n = 25)	17.2 \pm 1.2 \times 14.8 \pm 1.0	14.9 \pm 1.2 \times 12.5 \pm 0.8	15.9 \pm 1.8 \times 12.8 \pm 1.4
1-celled teliospore body (n = 10)	28.4 \pm 3.8 \times 15.5 \pm 1.3	27.0 \pm 3.4 \times 15.7 \pm 1.5	30.1 \pm 3.5 \times 18.2 \pm 2.8
1-celled teliospore stalk (n = 10)	6.1 \pm 0.9	5.5 \pm 0.9	5.5 \pm 0.8
2-celled teliospore body (n = 25)	40.7 \pm 4.6 \times 20.9 \pm 2.6	41.6 \pm 4.1 \times 19.5 \pm 1.7	42.5 \pm 6.7 \times 19.1 \pm 2.6
2-celled teliospore stalk (n = 25)	6.5 \pm 1.2	5.4 \pm 0.8	5.7 \pm 0.9

Figure 3: *Puccinia lagenophorae* on *Senecio vulgaris*. (A) Whole plant with aecial infections on stem and leaves. (B) Aecia and telia on stem. (C) Aeciospores (left) and teliospores (right).

Slika 3: *Puccinia lagenophorae* na *Senecio vulgaris*. (A) Celotna rastlina z ecijskimi okužbami na stebelu in listih. (B) Eciji in teliji na stebelu. (C) Eciospore (levo) in teliospore (desno).

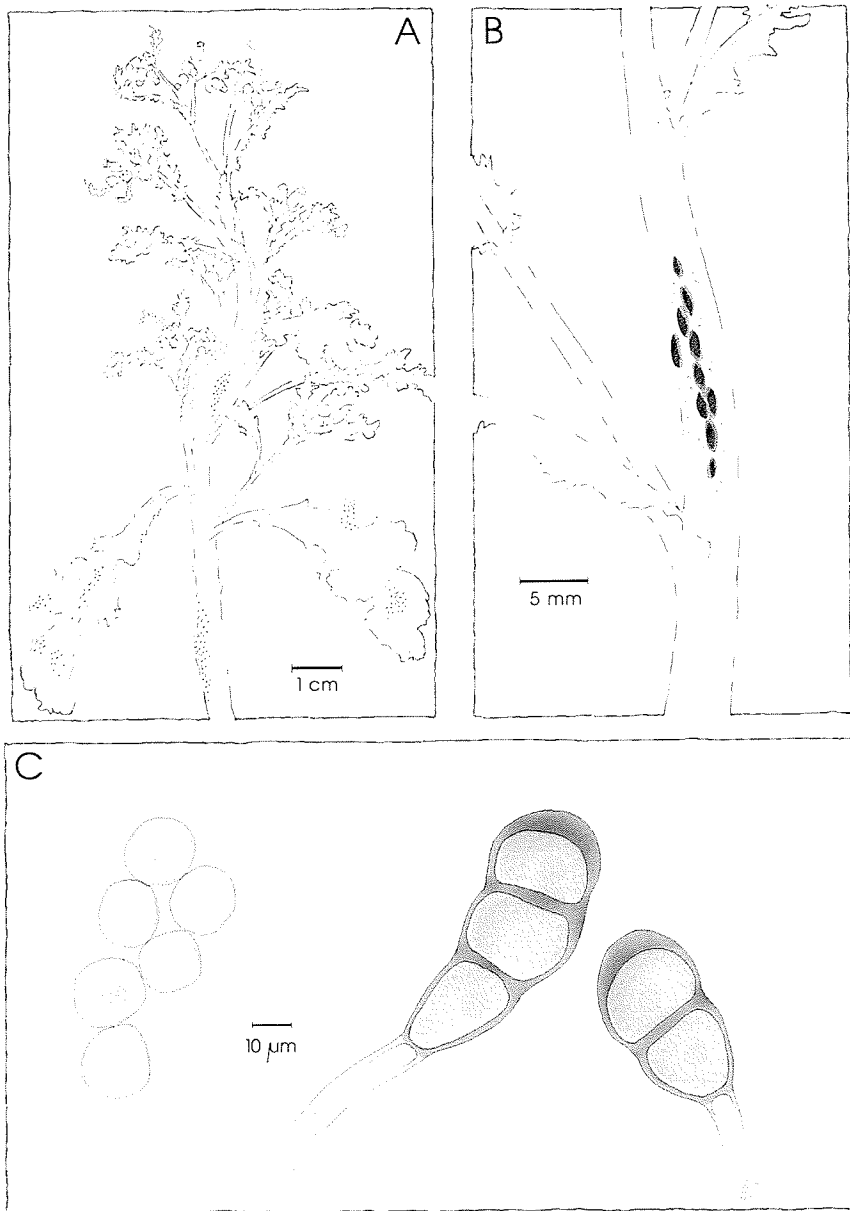
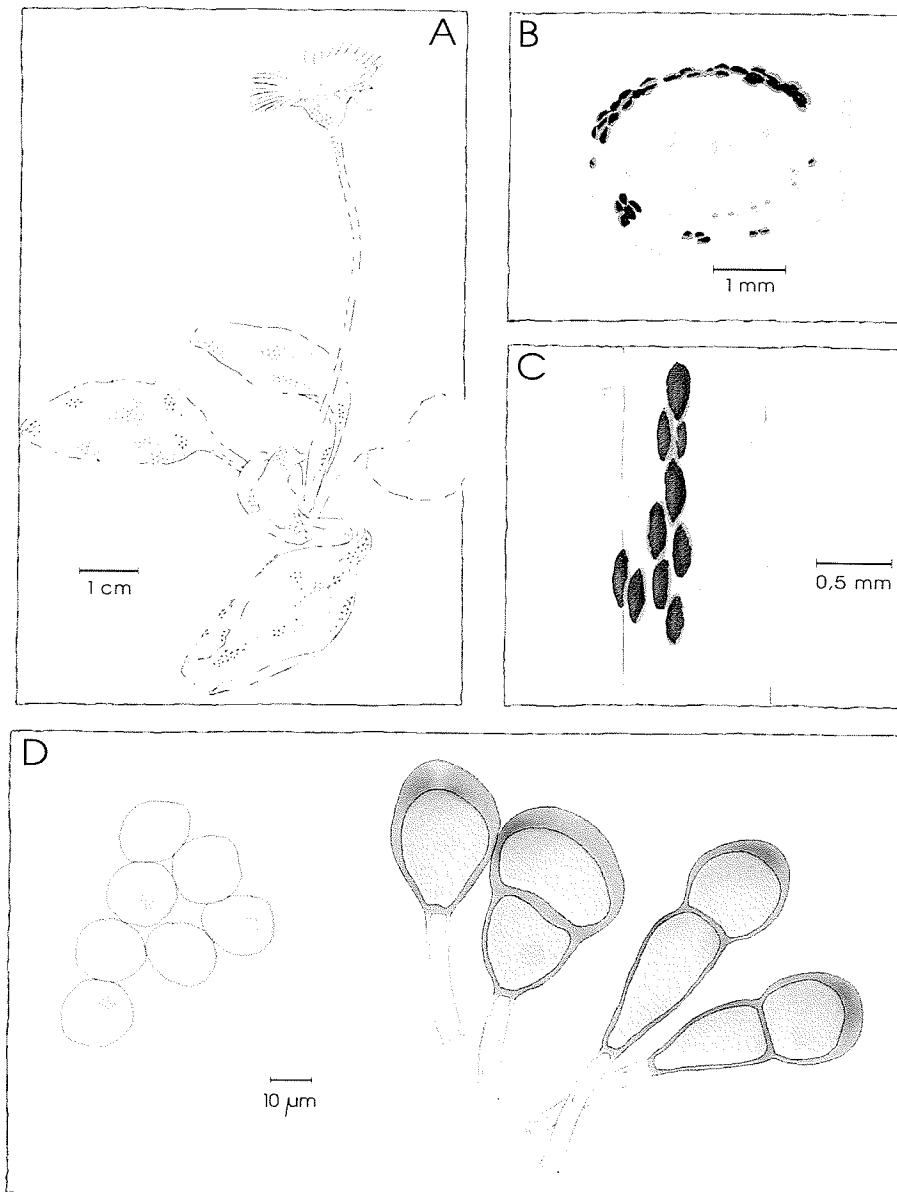


Figure 4: *Puccinia distincta* on wild *Bellis perennis*. (A) Whole infected plant with upright, curled leaves. (B) An infection lesion with discrete aecia and the dark confluent telia, on adaxial leaf surface. (C) Telia on inflorescence. (D) Aeciospores (left) and teliospores (right). Note the narrow teliospore stalks.

Slika 4: *Puccinia distincta* na negojenih *Bellis perennis*. (A) Celotna rastlina s pokončnimi, zvitimi listi. (B) Okužba s posamičnimi eciji in temnimi, zraščeni teliji na spodnji strani lista. (C) Teliji na cvetnem stebelu. (D) Eciospore (levo) in teliospore (desno). Opazni so ožji podstavki teliospor.



4. DISCUSSION

The present paper appears to be the first report for Slovenia of two rusts on Asteraceae, *Puccinia lagenophorae* on *Senecio vulgaris* and *P. distincta* on *Bellis perennis*. Whereas the former invaded Europe some 40 years ago and spread rapidly thereafter (Viennot-Bourgin, 1964; Scholler, 1994), the daisy rust epidemic has started only relatively recently (Preece *et al.*, 2000). Our observations, too, indicate that *P. lagenophorae* is well-established in Slovenia, being found in all parts of the country and in most *S. vulgaris* populations sampled. Further, this rust fungus was reported from Croatia as early as 1984 (Müller, 1995). In contrast, the incidence of rust infections on *B. perennis* was patchy in Slovenia, being focused mainly on the western part of the country. Further, within infected populations, discrete foci of infection were frequently observed, hinting at the very recent arrival of *P. distincta* in Slovenia. In fact, based on our own observations in spring 2000 (Jurc & Weber, 2000) which located *P. distincta* at a coastal site in Croatia but failed to find it in Slovenia despite careful searches, we believe that this pathogen probably arrived in Slovenia only in early summer of the year 2000. This recent date of arrival would be in line with the successive first records of the epidemic in France, Britain, Germany, Italy, the Czech Republic, Hungary and Croatia from 1996 onwards (Scholler, 1997; Gullino *et al.*, 1999; Jurc & Weber, 2000; Müller, 2000; Preece *et al.*, 2000).

Despite its recent arrival, however, *P. distincta* already appears to have become firmly established in Slovenia. The first ripe aecia of the year 2001 were seen on wild *B. perennis* in Ljubljana on 18 March, and within one week infections had become heavy and widespread (D. Jurc, unpublished observations).

Although *P. lagenophorae* and *P. distincta* are morphologically very similar, Weber *et al.* (1998 *b*) have distinguished them on the basis of infection experiments and teliospore morphology, notably the presence of three-celled teliospores only in *P. lagenophorae* which also had a significantly wider teliospore stalk. These differences were observed with English material but have subsequently been confirmed with samples collected in Germany (R. W. S. Weber, unpublished), the Czech Republic (Müller, 2000) and Slovenia (present paper). The presence of healthy *B. perennis* populations in the immediate vicinity of rusted *S. vulgaris* at several locations in Slovenia in 2000 lends further support to the distinct identity of these two rusts, and also to the notion that *P. distincta* must be a very recent arrival in Slovenia.

Data such as those reported here are relevant not only in the epidemiological context within which they were obtained, but they may be useful also for applied purposes, notably an evaluation of the suitability of *P. lagenophorae* as a bioherbicide against *S. vulgaris*. In this respect, an indication that *P. lagenophorae* may not be the cause of the current epidemic on *B. perennis*, one of the most widespread wild plants of Europe as well as a significant spring bedding plant, is encouraging.

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