NAT. CROAT. | VOL. 9 | No 4 | 225–236 | ZAGREB | December 31, 2000

ISSN 1330-0520
UDK 582.285.2˙581.137.3/582.998(497.5)

original scientific paper / izvorni znanstveni rad

FIRST REPORT OF *PUCCINIA DISTINCTA* MCALPINE, THE NEW EUROPEAN RUST ON DAISIES (*BELLIS PERENNIS* L.), FROM CROATIA

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Jurc, D. & Weber, R. W. S.: First report of *Puccinia distincta* McAlpine, the new European rust on daisies (*Bellis perennis* L.), from Croatia. Nat. Croat., Vol. 9, No. 4., 225–236, 2000, Zagreb.

Severe rust infections on wild and cultivated daisies (*Bellis perennis* L.) were found in April and May 2000 in an isolated coastal location of Croatia between Lovran and Opatija. The pathogen was identified as *Puccinia distincta* McAlpine which has been spreading rapidly across Europe for the past four years and may be of Australian origin. In the current paper, we describe the diagnostic features of *P. distincta* using Croatian material, and suggest spraying with myclobutanil-containing fungicides to protect ornamental daisies in flowerbeds. Without such treatments, it may no longer be possible to grow daisies in areas affected by *P. distincta.*

Key words: aeciospores, *Bellis perennis*, daisy, *Luzula*, myclobutanil, *Puccinia distincta*, *Puccinia lagenophorae*, *Puccinia obscura*, rust, *Senecio*, teliospores

Jurc, D. & Weber, R. W. S.: Prvo priopćenje o vrsti *Puccinia distincta* McAlpine, novoj europskoj hrđi na tratinčicama (*Bellis perennis* L.) iz Hrvatske. Nat. Croat., Vol. 9, No. 4., 225–236, 2000, Zagreb.

Teška infekcija hrđe na divljim i uzgajanim tratinčicama (*Bellis perennis* L.) otkrivena je u travnju i svibnju 2000. na izoliranom priobalnom području između Lovrana i Opatije. Patogena gljiva bila je determinirana kao *Puccinia distincta* McAlpine, koja se protekle četiri godine brzo širi po Europi, a možda dolazi iz Australije. U ovom prikazu opisujemo dijagnostičke značajke gljive *P. distincta* primjenjujući materijal iz Hrvatske i predlažemo prskanje fungicidima na osnovi miklobutanila kako bismo zaštitili dekorativne tratinčice u cvjetnim nasadima. Bez takvih tretmana postoji mogućnost da na područjima zaraženim s *P. distincta* više neće biti moguć njihov uzgoj.

Ključne riječi: *Bellis perennis*, ecidiospora, *Luzula*, miklobutanil, *Puccinia distincta*, *Puccinia lagenophorae*, *Puccinia obscura*, hrđa, *Senecio*, teleutospora, tratinčica

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INTRODUCTION

A rust disease of epidemic proportions is currently spreading across Europe on wild as well as cultivated forms of the daisy (*Bellis perennis* L.). Infections of ornamental daisies can cause such rapid and widespread mortality of the host that daisies have been abandoned as spring bedding plants by some local authorities in areas affected by the epidemic. The pathogen, *Puccinia distincta* McAlpine (WEBER *et al.*, 1998*a*), is a short-cycled rust which spreads by means of aeciospores capable of re-infecting the same host species on which they were produced (GROVE, 1913). This feature, which is unusual among rust fungi, clearly contributes to the rapid spread of the disease once it has become established. After prolonged infection, teliospores may also be formed. These can be germinated experimentally, but the basidiospores thus produced are not infective on *B. perennis* so that the role of the teliospores in the life-cycle is uncertain (Fig. 1; WEBER *et al.*, 1998*b*). It is possible that teliospores of *P. distincta* are a redundant relic from an ancestral species in which they were involved in sexual reproduction. With its aeciospores being the only remaining infective spore stage, *P. distincta* may be considered an anamorphic fungus (see SAVILE, 1979).

Puccinia distincta is thought to be derived from the macrocyclic *P. obscura* J. Schröt. which develops pycnia and aecia on *B. perennis*. However, aeciospores of *P. obscura* cannot re-infect the aecial host and instead cause infection on a second, taxonomically unrelated host (*Luzula* spp.) on which urediniospores and teliospores are produced (Fig. 2; GROVE, 1913). *Puccinia obscura* has been long-established in Europe (PLOWRIGHT, 1889; GROVE, 1913; BRANDENBURGER, 1985), but it does not cause serious infections of daisies presumably due to the inability of its aeciospores to re-infect *B. perennis*, and because it requires its two host species to be present in close proximity.

If *P. distincta* really originated from *P. obscura*, this evolutionary event probably took place place in Australia where *P. distincta* was first described as a serious threat to daisy growing about 100 years ago (MCALPINE, 1906). An examination of herbarium material at Kew Gardens revealed that *P. obscura* was also present in Australia at that time (WEBER *et al.*, 1998*b*), as were daisies which had been introduced from Europe in the mid-19th century (GROVE, 1913). Furthermore, the teliospores of *P. distincta* on *B. perennis* and of *P. obscura* on *Luzula* spp. are morphologically indistinguishable from one another. Accordingly, *P. distincta* must be a relatively recent re-introduction into Europe or it may have arisen here from *P. obscura* on a separate occasion. *Puccinia distincta* can be distinguished from *P. lagenophorae* Cooke, another short-cycled rust pathogen of Compositae (especially *Senecio* spp.) introduced from Australia (WILSON *et al.*, 1965; SCHOLLER, 1994; MÜLLER, 1995), by differences in teliospore morphology and by the absence of pycnia which are sometimes produced by *P. lagenophorae* (WEBER *et al.*, 1998*b*).

The first reports of the European daisy rust epidemic caused by *P. distincta* have appeared only very recently (SCHOLLER, 1997; WEBER *et al.*, 1998*a*), even though it may be reconstructed that serious infections on cultivated daisies have been occurring since at least 1993 in Germany (SCHOLLER, 1997) and possibly as early as 1981

in Britain (PREECE *et al.*, 2000). The current epidemic is probably due to a new strain of *P. distincta* with an enhanced ability to infect the ubiquitous wild *B. perennis*, thus creating a vast inoculum reservoir (PREECE *et al.*, 2000). Verified records of widespread infections of wild daisies due to *P. distincta* have been made across Southern and Central England (since April 1997), in Germany (Berlin, Oct. 1997;

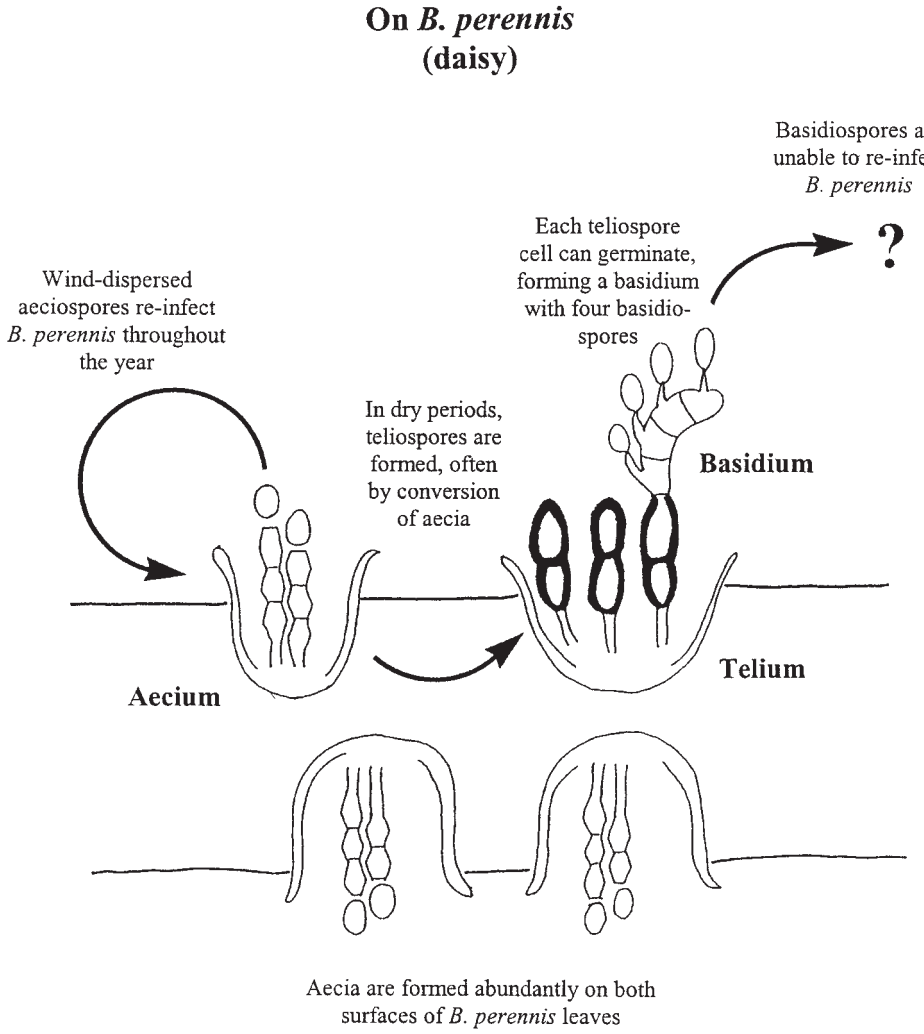


Fig. 1. Life-cycle of *Puccinia distincta* McAlpine. *Puccinia lagenophorae* Cooke has a similar life-cycle with aeciospores and teliospores on *Senecio* spp., but it also produces pycnia which have not been observed in *P. distincta*. After WEBER *et al.* (1998*b*).

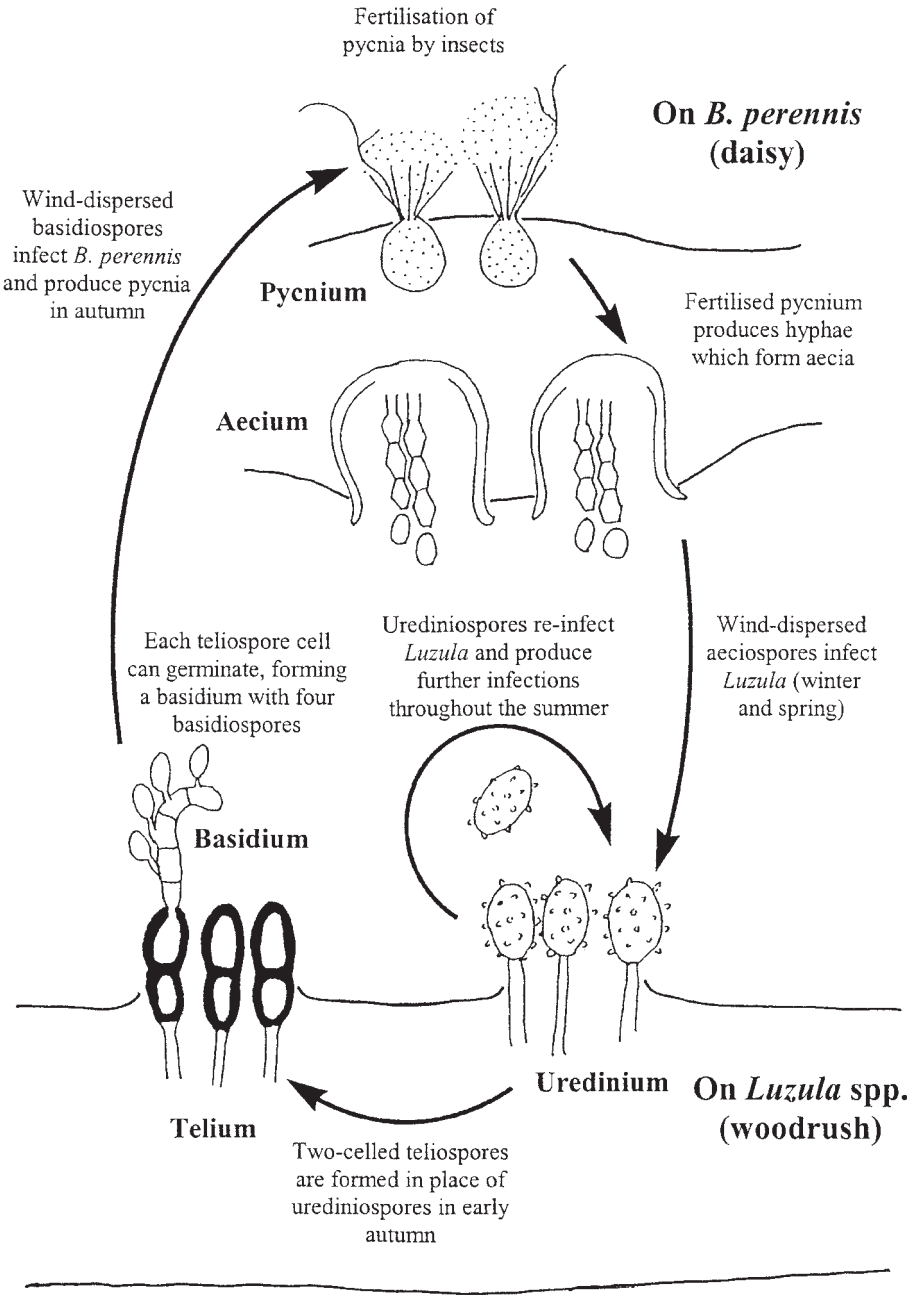


Fig. 2. Life-cycle of *Puccinia obscura* J. Schröt. This macrocyclic heteroecious species needs to alternate between two host species in order to complete its life-cycle. After WILSON & HENDERSON (1966).

Konstanz, July 1998; Buxtehude nr. Hamburg, Sept. 1999), the Czech Republic (Prag, Sept. 1998), Hungary (Budapest, July 1998), and France (Belfort, Oct. 1996; Boulogne, Sept. 1999; Perpignan, Oct. 1999).

In the present contribution, we report the first sighting of the daisy rust in Croatia, at an isolated coastal location between Lovran and Opatija, where it was found on wild as well as cultivated daisies. The pathogen has been identified as *P. distincta*. Further, we discuss the availability of fungicide treatments as effective control measures to protect ornamental daisies in flowerbeds.

MATERIALS AND METHODS

Flower beds with ornamental daisies (cultivar Pomponette) and lawns containing populations of wild *B. perennis* were inspected on 26–29 April and 25 May 2000 in a 6 km stretch between Opatija and Lovran from the sea shore to about 500 m inland. Further observations on wild *B. perennis* were made elsewhere in Croatia (Matulji and Jurdani) and in Slovenia (Jelšane, Ilirska Bistrica and Ljubljana). Infected plants were investigated *in situ* for macroscopic symptoms of the disease, and herbarium material was collected and has been deposited at the Herbarium of the Slovenian Forestry Institute.

For microscopic measurements, aeciospores were collected by careful brushing of ripe aecia, and mature spores were identified by the presence of numerous small instead of one or a few large oil droplets. Teliospores and mesospores were removed from telial lesions with a thin needle. The dimensions of aeciospores, peridial cells and two-celled teliospores (25 measurements per sample) and of one-celled mesospores (10 measurements per sample) were determined to within 0.5 µm accuracy. Samples of all spore types were measured from infected plants collected at three different locations (see Tab. 1). Representative spores were photographed with a Zeiss Axioskop 2 light microscope using differential interference contrast optics.

RESULTS

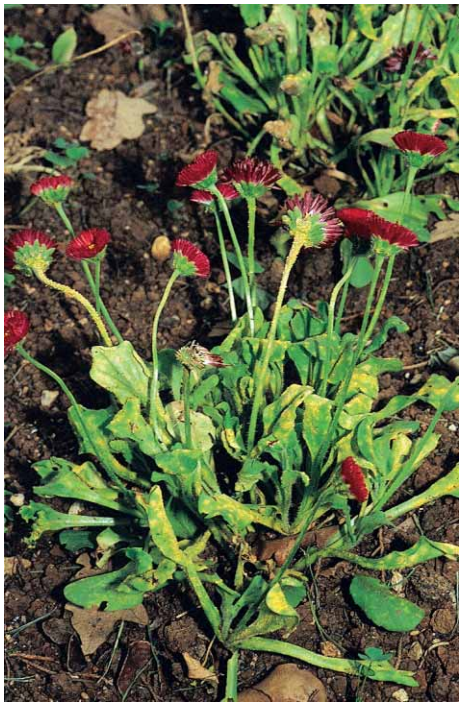
Profuse infections of wild and cultivated daisies were found during both sampling periods at all inspected sites between Lovran and Opatija, two towns in close geographical proximity to each other at the Adriatic coast in the north of Croatia (quadrants 0651/4 and 0751/2). No evidence of the disease was found elsewhere in Croatia or in Slovenia. A summary of all locations investigated is given in Fig. 11.

Diseased plants showed symptoms typical of *P. distincta* (Figs 3 and 4), beginning with the development of an uneven leaf surface due to the enlargement of underlying aecia, followed by breaking of the epidermis. Aecia were approx. 0.15–0.2 mm in diameter. The bright yellowish-orange aeciospores were contained within a protruding white peridium (Fig. 5). In individual infections, aecia were arranged in concentric rings within yellowing areas on both sides of the leaf whereas in heavy infections, aecia were distributed over the entire leaf surface. More than 500 indi-

vidual aecia were commonly produced on a single infected leaf. Such severe infections were characterised by a distortion and chlorosis of leaves which were also in a more upright position (Fig. 3). Aecia also occurred abundantly on inflorescences (Fig. 4), involucral bracts and even on the flower axis among unripe seeds.

The development of black elongated telia (Fig. 6) occurred only on severely diseased plants and was noticeably more common on wild than on cultivated daisies. Telia were most abundant at the base of inflorescences and petioles as well as abaxial midribs of leaves, but they were very rare on leaf blades where aecia occurred abundantly. No pycnia or other rust spore stages were observed.

Infections of *P. distincta* on cultivated daisies between Lovran and Opatija were particularly devastating, resulting in moribund plants without ornamental value in late April, and uprooting of the dead plants by 25 May 2000. Gardeners from the Parkovi Opatija company reported that ornamental daisies had been planted there for several previous years without any noticeable rust infection, the only previous incidence of daisy mortality having been attributed to damping off caused by *Fusarium* spp. In view of the damage caused by *P. distincta*, ornamental daisies may not be used in future plantings by the company.

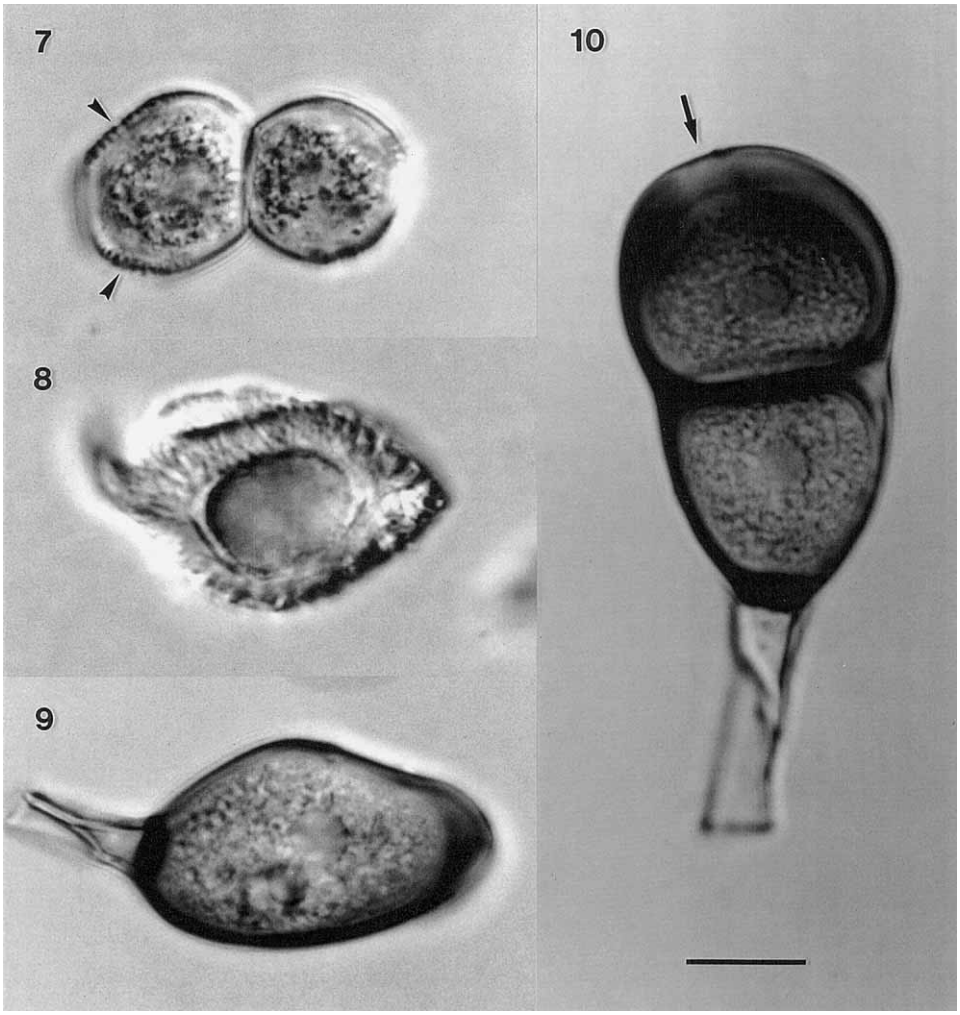


Figs 3 and 4. Heavy aecial infections of *Puccinia distincta* McAlpine on an ornamental *Bellis perennis* L. plant (cultivar Pomponette), Lovran, 26 April 2000. **Fig. 3.** Whole plant showing the reduced ornamental value caused by the infection. **Fig. 4.** Aecial infection on an inflorescence.

Figs 5 and 6. Infections of *P. distincta* on wild *B. perennis*, Lovran, 27 April 2000. **Fig. 5.** Abaxial leaf midrib showing aecia, each delimited by a protruding whitish peridium. Dust-like yellowish-orange aeciospores have been released. A black elongated telium is also visible. **Fig. 6.** Several telial lesions on an inflorescence.

Among wild daisies, the disease was prominent in shady places, e.g. under trees, bushes or in the shade of houses, as well as on well-watered lawns. It was infrequent on or absent from daisies growing in sunny and dry locations.

Microscopically, aeciospores (Fig. 7) were found to be spheroidal or slightly angular in outline, with a hyaline but minutely roughened wall. The cytoplasm of mature aeciospores contained numerous small lipid droplets, that of young ones a few large drops. A bright yellowish pigment was localised within these lipid droplets. The sterile cells making up the peridial wall of the aecium were lozenge-shaped and characterised by a thick roughened cell wall (Fig. 8). Within telial lesions, a certain proportion (up to 25%) of one-celled spores (mesospores; Fig. 9) was found, in addition to the typical two-celled teliospores (Fig. 10). Both spore types were borne on a hyaline, cylindrical stalk which collapsed at maturity. Three-celled teliospores were not observed. The teliospore wall was heavily thickened especially at the top of the upper cell and contained a dark brown pigment. A canal or germ pore was occasionally visible, traversing the apical cell wall in its thickest region (Fig. 10).



Figs 7–10. Material of *Puccinia distincta* McAlpine from an infected ornamental *Bellis perennis* L. plant (cultivar Pomponette), collected in Lovran on 26 April 2000. Scale bar = 10 µm in all cases. **Fig. 7.** Two aeciospores. Note the pigmented lipid droplets and the slightly roughened spore wall (arrowheads). **Fig. 8.** Lozenge-shaped peridial cell with a very thick, roughened wall. **Fig. 9.** One-celled mesospore. **Fig. 10.** Typical two-celled teliospore with a thick, pigmented wall and a hyaline collapsed stalk. Note the germination pore through the thick wall layer of the upper cell (arrow).

Spore measurements (Tab. 1) revealed an average size of 16.6 × 14.3 µm (range 14–23 × 11–17 µm) for aeciospores, 31.1 × 16.2 µm (range 25–40 × 11–22 µm) for peridial cells, 39.7 × 19.5 µm (range 30–50 × 16–23 µm) for two-celled teliospores and

26.8 × 16.5 µm (range 18–32 × 13–21 µm) for one-celled mesospores. The average width of the teliospore stalk at the point of attachment to the lower cell was 6.1 µm (range 4.0–8.0 µm) for teliospores and 5.6 µm (range 4.0–7.5 µm) for mesospores.

Tab. 1. Spore dimensions in three samples of *Puccinia distincta* L. collected from Croatia. For details, see below.

	Sample 1	Sample 2	Sample 3
Aeciospores	16.3 ± 2.4 × 13.4 ± 1.2	16.8 ± 1.6 × 14.2 ± 1.8	16.8 ± 1.3 × 15.3 ± 1.3
Peridial cells	34.8 ± 4.3 × 16.3 ± 3.3	29.8 ± 2.7 × 17.3 ± 2.3	28.8 ± 3.6 × 14.9 ± 1.9
Teliospores	37.0 ± 3.4 × 19.3 ± 1.4	41.8 ± 4.7 × 19.4 ± 2.1	40.2±5.0 × 19.8±1.9
Teliospore stalks	6.5 ± 0.9	5.8 ± 0.7	5.8 ± 1.1
Mesospores	26.0 ± 2.5 × 16.8 ± 1.3	27.7 ± 5.6 × 15.4 ± 2.4	26.7 ± 1.4 × 17.4 ± 1.6
Mesospore stalks	6.1 ± 0.7	5.1 ± 0.6	5.6 ± 1.2

All values are given in µm as the average ± standard deviation of 10 measurements (mesospores and mesospore stalks) or 25 measurements (all other data). Samples were collected as follows:- Sample 1, ornamental *B. perennis* (cultivar Pomponette) from Lovran, Hotel Excelsior, 26 April 2000; Sample 2, wild *B. perennis* from Lovran, lawn, 25 May 2000; Sample 3, wild *B. perennis* from Opatija, Margarita Park, 25 May 2000.

DISCUSSION

The macroscopic as well as microscopic features of the rust infection on wild and cultivated *B. perennis* observed in the vicinity of Opatija and Lovran correlate well with those reported for *P. distincta* in the literature (WEBER *et al.*, 1998*a*,*b*). On the basis of several key features, the pathogen could be distinguished from *P. lagenophorae*, the rust on *Senecio* spp. which may (WILSON *et al.*, 1965) or may not (WEBER *et al.*, 1998*b*) be able to infect daisies. Firstly, one-celled mesospores were present among the usually two-celled teliospores but three-celled spores, typically also found in *P. lagenophorae*, were absent. Secondly, the point of attachment of the stalk to the teliospore was relatively narrow (average 6.1 µm), which corresponds well with the value of 6.6 µm previously determined for *P. distincta* but contrasts with the stalk width of 8.4 µm measured for *P. lagenophorae* (WEBER *et al.*, 1998*b*). Finally, given that *P. lagenophorae* was reported from Croatia (Istria) as early as 1984 (MÜLLER, 1995), one would have expected rust infections especially on cultivated *B. perennis* to break out at that time if they were caused by cross-infections of *P. lagenophorae* from *Senecio* spp. Altogether, our results leave little doubt that the incidence of daisy rust in Croatia is caused by *P. distincta*, and that the current European daisy rust epidemic has therefore reached Croatia. A similar epidemic on *B. perennis* has recently been reported from neighbouring regions of Italy by GULLINO *et al.* (1999), although these authors attributed the disease to *P. lagenophorae.* Since, in all probability, the rust on *B. perennis* has arrived in Croatia only very recently, it would be very interesting to perform a thorough investigation of its distribution and to monitor its current spread, as well as that of *P. lagenophorae* on *Senecio* spp., across the whole of Croatia.

Fig. 11. Distribution of *Puccinia distincta* McAlpine on wild and cultivated forms of *Bellis perennis* L. in Croatia, April to May 2000. Presence (●) or absence (○) of the pathogen are indicated. All sites examined are indicated except for Ljubljana, where no daisy rust was found.

One intriguing observation was the far greater abundance of teliospores on wild as compared to cultivated forms of *B. perennis*, and their presence primarily on longer-lived plant organs such as petioles or inflorescences rather than the rapidly-wilting leaf blades. During greenhouse experiments with cultivated daisies, WEBER & TILSTON (1999) found that the plants were killed in less than 7 weeks after artificial inoculation and showed only aecial symptoms. However, when mild fungicide treatments were applied to prolong the lives of the plants by as little as 3–4 weeks, telia were also formed (R. W. S. WEBER, unpublished). Therefore, cultivated daisies are probably so highly susceptible to *P. distincta* that they succumb before the fungus has produced teliospores whereas infections on wild daisies progress more slowly, so that telia can develop.

In view of the high susceptibility of cultivated daisies to *P. distincta* and the rapid spread of the fungus across Europe, the main aim of the greenhouse trials mentioned above was to find a suitable fungicide for the control of the disease. Myclobutanil showed excellent protective and curative effects (WEBER & TILSTON, 1999; see also GULLINO *et al.*, 1999) and has since been applied successfully in field situations in Britain. Indeed, this active ingredient is so effective against *P. distincta* that

it has saved at least one commercial cultivar of *B. perennis*, of which only a few heavily infected plants were left at the time of the first spray. In Croatia, myclobutanil is available in several formulations, including Systhane 6 FLO (active ingredient, myclobutanil), Systhane 12 E (a.i., myclobutanil), Systhane MZ (a.i., myclobutanil and mancozeb) and Sabithane (a.i., myclobutanil and dinocap), all produced by Rohm and Haas and marketed by Chromos (ANON., 2000). We recommend an early spray as soon as the first aecial lesions appear, and 2–4 repeats at fortnightly intervals. With this schedule, it should be possible to maintain ornamental daisies disease-free throughout the growing season.

Experience from other European countries shows that *P. distincta* persists once it has arrived and become established. The fungus probably survives the winter as latent or aecial infections on the ubiquitous wild daisy. In southern England, in each of the past 4 years there has been a dramatic surge in the abundance and severity of infections starting from April to June, depending on weather conditions, whereby the outbreak of an epidemic seems to be favoured by high temperature.

In view of the persistent nature of the daisy rust epidemic, it would seem worthwhile to initiate breeding programmes for increased resistance of ornamental daisies against *P. distincta*, so that the application of fungicides can be abandoned or at least reduced in frequency in future.

ACKNOWLEDGEMENTS

We thank Miss M. Đeldum and her colleagues of the Parkovi Opatija company for useful discussions, Prof. J. Webster for help with the conception and drawing of the life-cycles, Prof. T. Wraber for helpful discussions, and Miss I. Tavčar and Mr. T. Kralj for drawing the map. Finally, we are grateful to Dr. H. B. Gjærum, Prof. K. Mendgen, Dr. T. F. Preece, Miss E. L. Tilston, Miss F. Weber and Prof. J. Webster for sending us specimens of infected wild *B. perennis* from various parts of Europe.

Received July 15, 2000

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SAŽETAK

Prvo priopćenje o vrsti *Puccinia distincta* McAlpine, novoj europskoj hrđi na tratinčicama (*Bellis perennis* L.) iz Hrvatske

D. Jurc & R. W. S. Weber

Jaki napad hrđe koja oštećuje divlje i uzgojene tratinčice (*Bellis perennis* L.) zapažen je u travnju i svibnju 2000. na lokaliziranom području između Lovrana i Opatije. Bolest nije pronađena drugdje u Hrvatskoj. Značajke ove bolesti su brojne svjetlonarančaste ecidije u obliku čaše (oko 0,15–0,2 mm u promjeru) na objema stranama lista, a i na peteljkama listova i na cvatovima. Teško zaraženi listovi su bili uspravni, savinuti i požutjeli, nakon toga su odumrli. Ecidijske zaraze prouzrokovale su veliko odumiranje posebno onih tratinčica koje se uzgajaju u cvjetnim nasadima. Nakon dugotrajne zaraze povremeno su nastajale crne teliospore, naročito na divljim tratinčicama. Uzročnik sadašnje epidemije hrđe na *B. perennis* diljem Europe bio je determiniran kao *Puccinia distincta* McAlpine. Smatra se da je ta vrsta porijeklom iz Australije, a možda proizlazi iz *P. obscura* J. Schrot, makrocikličke hrđe koja se izmjenjuje između *B. perennis* i *Luzula*, koja je poznata u Europi od 19. stoljeća i nikad nije prouzrokovala teške bolesti na tratinčicama. Uzrok sadašnje epidemije *P. distincta* može se bar djelomično pripisati razvitku novog soja gljive s povećanom sposobnošću zaraze divljih tratinčica. *Puccinia distincta* može se efikasno kontrolirati prskanjem fungicidima na osnovi miklobutanila.