

# Epidemic increase of *Endophyllum osteospermi* (Uredinales, Pucciniaceae) on *Chrysanthemoides monilifera*

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## Abstract

The epidemiology of the rust fungus *Endophyllum osteospermi* was investigated. This rust fungus is considered to be a candidate biological control agent for *Chrysanthemoides monilifera* ssp. *monilifera*, which is an invasive alien weed of native vegetation in south-eastern Australia. Between 10 and 20 plants of *C. monilifera* were marked at each of five sites in the Western Cape Province of South Africa, where both organisms are native. The infection levels and number of witches' brooms were determined every 2 months over a 2-year period. Additionally, at three of these sites, the infection levels and number of witches' brooms of all bushes in the host population was determined annually over 4 years. The increase in number of witches' brooms per bush ranged between 0 and 282 within 1 year, with an average increase per bush of 28 (SE±4.8) in 1993, and 39 (SE±9.2) in 1994. The average  $r_s$  for all bushes during 1993 was 0.015 month<sup>-1</sup> (SE±0.0041,  $n=72$ ) and 0.0098 month<sup>-1</sup> (SE±0.0073,  $n=43$ ) during 1994. When host bushes that either died back or died during the course of each year were excluded, then the average  $r_s$  during 1993 was 0.023 month<sup>-1</sup> (SE±0.0048,  $n=45$ ) and 0.0348 month<sup>-1</sup> (SE±0.0106,  $n=20$ ) during 1994. Under suitable conditions in South Africa, *E. osteospermi* undergoes epidemic increase within its host plant's populations. This rust fungus should therefore be considered as a suitable candidate biological control agent for use in Australia against *C. monilifera* ssp. *monilifera*.

**Keywords:** Alien invader, biological control, boneseed, environmental weed, natural pathosystem

## Introduction

Selection of successful candidate classical biological control agents in their native range is fraught with difficulties. Candidate agents should be damaging under natural conditions, in addition to being host specific (Watson 1991). Candidate agents in their native range should also preferably have a high incidence and be generally widespread on their host plants (Evans 2000). In the case of several highly successful classical biological control programmes using rust fungi, selection of the agents were based

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primarily on field observations that revealed the pathogens to be widespread and occurring at high incidences in their natural range. Subsequent to release, they rapidly increased and reduced their weed host's density. Examples include *Maravalia cryptostegiae* (Cummins) Y. Ono. released in Australia (Evans 1993, 2000; Vogler et al. 2002), *Puccinia chondrillina* Bubák & Syd. (Hasan & Wapshere 1973) employed in Australia (Burdon et al. 1981) and the USA (Supkoff et al. 1988), and *Uromycladium tepperianum* (Sacc.) McAlpine used in South Africa (Morris 1991, 1997).

Unfortunately, anticipated success has not always been achieved. The perennial systemic rust fungus *Puccinia punctiformis* (F. Strauss) Röhl. can cause reduced growth and reproduction (Thomas et al. 1994) and high mortality of infected *Cirsium arvense* (L.) Scop. plants (Watson & Keogh 1981), and has long been considered a potential biocontrol agent (Frantzen 1994). An investigation into its incidence in its native range demonstrated that this rust fungus generally occurred at low incidences (1–14% in four naturally infected populations over 2 years), though it was recorded having localised foci of infection with high incidence (Frantzen 1994). It was therefore concluded that this rust fungus species would have to be used in an augmentative programme for it to be a successful biocontrol agent (Frantzen 1994).

The autoecious, microcyclic rust fungus *Endophyllum osteospermi* (Doidge) A.R. Wood causes systemic infections, resulting in witches' brooms on *Chrysanthemoides monilifera* (L.) Norl. ssp. *monilifera* (Asteraceae, Calenduleae) (Morris 1982). This host plant is a woody, perennial bush that is common in the Western Cape Province of South Africa (Norlindh 1943). Abundant aecidioid telia are produced on the witches' brooms (Morris 1982). The aecidioid teliospores germinate to produce vesicle-like modified basidiospores, which germinate to directly penetrate epidermal cells of immature host leaves. Penetration takes up to 72 h after germination is initiated (Morris 1982). Initial development of the witches' brooms is between 5 and 24 months after penetration, but they can persist for several years (Wood et al. in press). This rust fungus may therefore be considered as being monocyclic within years. The rust fungus survives through unfavourable climatic conditions in the perennial witches' brooms. Infection by this rust fungus is associated with a reduction in growth and reproduction of host plants under field conditions, especially when a large proportion of the host bush consists of witches' brooms. The growth of healthy branches (without witches' brooms) on infected bushes was 26–81% less than that of healthy branches on uninfected bushes. The number of buds, flowering capitulae, fruiting capitulae, and drupes on individual healthy branches of infected bushes was 35–75%, 45–90%, 15–99%, and 15–90% less, respectively, than those on uninfected bushes (Wood 2002). Severely infected plants often die (Morris 1982; Nesar & Morris 1984).

*Chrysanthemoides monilifera* spp. *monilifera* invades coastal and inland districts of Victoria, South Australia, New South Wales and Tasmania in cool-temperate winter-rainfall areas (Stahle 1997). As the rust is primarily adapted to a temperate climate (Wood et al. in press), *E. osteospermi* is a candidate agent for the biological control of *C. monilifera* spp. *monilifera* in Australia (Scott & Adair 1995; Adair & Edwards 1996).

Field observations throughout the native range of *E. osteospermi* indicated that this rust fungus was either rare or absent in many populations of its host, despite being generally widespread, and having a high incidence and severity in certain host plant populations (Wood 2002; pers. obs.). Thus, although *E. osteospermi* has been shown to be potentially damaging to its host plant (Morris 1982; Wood 2002), its variable

incidence in its native range is of some concern. The present study was therefore aimed at determining if *E. osteospermi* increased its incidence and severity each growth season (i.e., epidemic increase), or remained at constant levels. The incidence and severity of *E. osteospermi* in selected, wild populations of *C. monilifera*, was determined during 1992 to 1995. The study was conducted in the Fynbos Biome in the winter rainfall region of the Western Cape Province of South Africa. This study provides a basis for comparison between a biocontrol agent in its natural and introduced range, if it is released in Australia in future.

## Materials and methods

### *Increase of witches' brooms on individual host plants*

Five populations of *E. osteospermi* naturally infecting *C. monilifera* were studied, namely: (1) Heuningberg Nature Reserve, Bredasdorp 34°32'S 20°02'E (*C. monilifera* ssp. *monilifera*); (2) De Hoop Nature Reserve, E of Bredasdorp 34°32'S 20°02'E (*C. monilifera* ssp. *monilifera*); (3) Potberg Education Centre, De Hoop Nature Reserve, E of Bredasdorp 34°23'S 20°32'E (*C. monilifera* ssp. *monilifera*); (4) near Mossel Bay 34°10'S 22°05'E (1993 only) (*C. monilifera* ssp. *pisifera* (L.) Norl.); and (5) between Bellvidere and Brenton-on-Sea, Knysna 34°05'S 23°00'E (*C. monilifera* ssp. *pisifera*).

The number of witches' brooms per plant on between 10 and 20 selected host plants were recorded from within each study population, every 2 months from January 1993 until November 1994. The proportional volume of each bush consisting of witches' brooms (infection level) was also determined on each occasion. Bushes were selected so that there was a range of infection levels from each locality at initiation of the observations.

The simple interest infection rate ( $r_s$ ) was calculated annually for the estimated proportion of witches' brooms per bush (infection level) using the formula:

$$r_s = \frac{1}{t_2 - t_1} \cdot \log_e \frac{1 - x_1}{1 - x_2}$$

where  $t$  = time and  $x$  = infection level, and  $t_1$  and  $t_2$  were January and November, respectively, and  $r_s$  was calculated to be expressed as per month (Vanderplank 1963; Zadoks & Schein 1979).

### *Rust incidence within host populations*

At three sites (De Hoop and Potberg, and another site 4 km NW of Scarborough, Cape Peninsula 34°12'S 18°23'E (*C. monilifera* ssp. *monilifera*)), the area occupied by each plant population was divided into 5 × 5-m quadrants and the position and relative size of each bush was recorded. The number of witches' brooms and infection level was determined for each bush annually from 1992 until 1995.

## Results

### *Increase of witches' brooms on individual host plants*

The larger the host bush, the higher the initial number of witches' brooms that were recorded. However, considerable variation was observed in the number of witches'

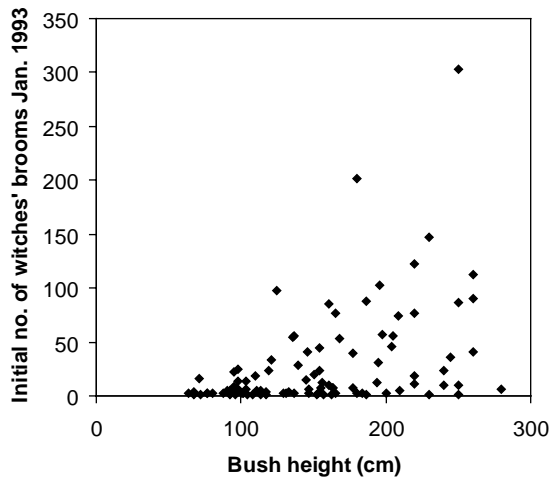


Figure 1. The number of witches' brooms caused by *Endophyllum osteospermi* on individual bushes of *Chrysanthemoides monilifera* at five sites in the Western Cape, South Africa, during January 1993.

brooms recorded throughout the size (height) range of the shrubs assessed (Figure 1). The increase in number of witches' brooms for each bush during both years varied greatly between bushes, between sites, and between years. The average increase in witches' brooms per bush was 28 ( $SE \pm 4.8$ , range of 0–282) in 1993, and 39 ( $SE \pm 9.2$ , range of 1–251) in 1994. The maximum number of witches' brooms recorded on any single host plant was 484 on a bush that initially had 202 witches' brooms, thus increasing by 282. This particular bush subsequently died during 1994. The maximum number of witches' brooms occurring in 1993 and 1994 against the initial

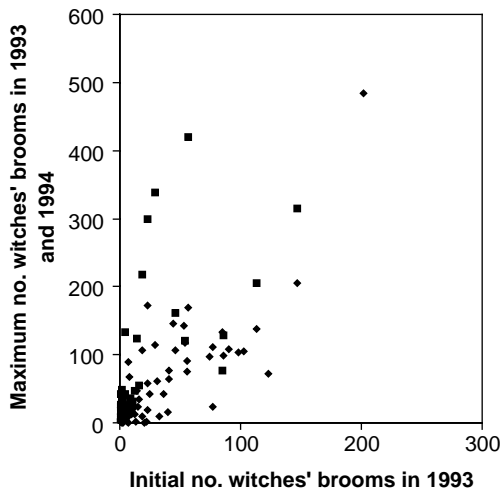


Figure 2. The maximum number of witches' brooms caused by *Endophyllum osteospermi* on marked bushes of *Chrysanthemoides monilifera* recorded during 1993 (diamonds) and 1994 (squares), against the initial number in January 1993, at five sites in the Western Cape, South Africa. Bushes that died or suffered die-back are excluded.

Table I. Total number of witches' brooms caused by *Endophyllum osteospermi* occurring on selected bushes in naturally infected populations of *Chrysanthemoides monilifera*

Year	Site	No. of bushes <sup>a</sup>	No. of witches' brooms					
			Jan.	Mar.	May	Jul.	Sep.	Nov.
1993	Bredasdorp	17	n.d. <sup>b</sup>	n.d.	165	295	429	542
	De Hoop	12	248	251	289	433	462	477
	Potberg	13	562	581	585	758	1276	1354
	Mossel Bay	8	182	195	335	351	388	449
	Bellvidere	13	356	400	348	446	497	558
1994	Bredasdorp	12	429	535	649	737	n.d.	822
	Potberg	5	421	525	594	733	1004	857
	Bellvidere	10	382	337	381	464	634	597

<sup>a</sup>Bushes that died or had die-back were excluded, total number of bushes monitored are indicated in Table II. <sup>b</sup>n.d., not determined.

number at the beginning of 1993 for each bush is shown in Figure 2. The total number of witches' brooms recorded at each site is given in Table I.

Some bushes suffered die-back, or died during the period of observation, with a far higher proportion of bushes exhibiting die-back during 1994 than in 1993. Die-back and death was in many cases associated with a high infection level of *E. osteospermi*. Of the 15 bushes at the Bredasdorp, De Hoop, Potberg, and Bellvidere sites that had infection levels of 50% or more in January 1993, three died and seven suffered severe die-back during the course of the 2 years of monitoring. Of eight bushes at the Mossel Bay and Potberg sites that had infection levels of 50% or more, three died and one suffered severe die-back during 1993.

Table II. Average simple interest rate of increase (month<sup>-1</sup>) of *Endophyllum osteospermi* on selected bushes of *Chrysanthemoides monilifera* at five sites in the Western Cape Province, South Africa, and the number of bushes with a positive, no, or negative change in severity of infection

Site	Year	Height of host bushes (cm) <sup>a</sup>	Total no. bushes	Change in infection level <sup>b</sup>			No. bushes died	Average $r_s$ ( $\pm$ SE) month <sup>-1</sup>
				+	0	-		
Bredasdorp	1993	(64 -) 104 (-220)	20	15	3	1	1	0.0127 ( $\pm$ 0.0053)
	1994		20	7	7	2	4	0.0137 ( $\pm$ 0.0101)
De Hoop	1993	(92 -) 165 (-260)	15	8	3	1	3	0.0146 ( $\pm$ 0.0102)
	1994		15	2	2	1	10	n.d. <sup>c</sup>
Potberg	1993	(114 -) 191 (-250)	10	7	3	0		0.0337 ( $\pm$ 0.0142)
	1994		10	6	2	2		0.037 ( $\pm$ 0.0165)
Mossel Bay	1993	(77 -) 150 (-240)	10	5	4	1		0.0254 ( $\pm$ 0.0152)
Bellvidere	1993	(71 -) 198 (-260)	17	5	9	3		-0.0007 ( $\pm$ 0.0067)
	1994		17	3	8	6		-0.0099 ( $\pm$ 0.0109)

<sup>a</sup>The (minimum-) average (-maximum) height of marked bushes. <sup>b</sup>Number of plants with a change in infection levels, where the infection level is the proportion of each bush consisting of witches' brooms caused by *E. osteospermi*, in classes of 5, 10, 25, 50, 75 and 100% infected; +, increase; 0, no change; -, decrease. <sup>c</sup>n.d., not determined.

Specifically at the De Hoop site, bushes began to die of an unknown cause during 1994, and the entire population had died by the end of 1996. This was not associated with high levels of infection by *E. osteospermi*, as was the above die-back and death of bushes. The 1994 data obtained from this site was therefore excluded from analysis.

The number of bushes of *C. monilifera* with an increase, no change, or a decrease in infection level is given in Table II. Except for the Bellvidere site, there were greater numbers of host bushes that had an increase in the number of witches' brooms rather than no increase or a decrease.

The average  $r_s$  for individual bushes during 1993 was  $0.015 \text{ month}^{-1}$  ( $\text{SE} \pm 0.0041$ ,  $n = 72$ , range  $-0.0693$  to  $0.1281 \text{ month}^{-1}$ ) and  $0.0098 \text{ month}^{-1}$  ( $\text{SE} \pm 0.0073$ ,  $n = 43$ , range  $-0.1609$  to  $0.1099 \text{ month}^{-1}$ ) during 1994. When host bushes that either died or died-back were excluded, then the average  $r_s$  during 1993 was  $0.023 \text{ month}^{-1}$  ( $\text{SE} \pm 0.0048$ ,  $n = 45$ , range  $0$  to  $0.1281 \text{ month}^{-1}$ ) and  $0.0368 \text{ month}^{-1}$  ( $\text{SE} \pm 0.0099$ ,  $n = 20$ , range  $0$  to  $0.1099 \text{ month}^{-1}$ ) during 1994.

### Rust incidence within host populations

The incidence of symptomatic bushes for each of the three sites is given in Table III. Incidence of witches' brooms increased at Scarborough (33–76.5%) and Potberg (13–48%), but remained approximately constant at the De Hoop site. There was a slight decrease in overall incidence at the Potberg site between 1994 and 1995, as there were approximately 55 newly recruited bushes. However, if the new recruits are excluded, then the incidence increased to 54%. At the Scarborough site, an outbreak of a tortricid moth (Lepidoptera, Tortricidae) occurred during 1993 and continued into 1994. A high host plant mortality was associated with the severe feeding damage, though the feeding may not be directly responsible for the mortality, as reflected by the decrease in number of host plants present.

Table III. Percentage of *Chrysanthemoides monilifera* ssp. *monilifera* bushes in three populations in the Western Cape Province, South Africa, naturally infected with *Endophyllum osteospermi* at various infection levels

Site	Year	Total no. bushes	No. infected bushes	No. witches' brooms <sup>a</sup>	Infection level classes <sup>b</sup>						
					0%	5%	10%	25%	50%	75%	100%
Scarborough	1992	244	79	303	67 <sup>c</sup>	25	5	2	1	0	0
	1993	200	152	999	23.5	40	25	10	1	0.5	0
	1994	36	25	234	31	17	31	19	3	0	0
De Hoop	1992	128	65	–	49	35	9	3	1.5	2 <sup>d</sup>	0
	1993	119	71	872	40	29	19	10	1	1 <sup>d</sup>	0
	1994	116	64	873	45	30	10	11	3	0	0
Potberg	1992	154	20	168	87	11	1	1	1	0	0
	1993	164	54	1463	67	23	7	5	2	0	0.5
	1994	169	81	2478	52	31	8	5	2	1	2
	1995	216	92	2276	57.5	26	6	7	3	0.5	2 <sup>e</sup>

<sup>a</sup>Total number of witches' brooms on all infected bushes. <sup>b</sup>Infection level classes determined as volume of host bush occupied by witches' brooms. <sup>c</sup>Expressed as percentage of total number of bushes per infection level. <sup>d</sup>Bushes died before the following year's monitoring. <sup>e</sup>Excludes five additional bushes in this class that died during the year before the mapping was carried out.

## Discussion

A candidate biological control agent should preferably be damaging to its host (Watson 1991) and be widespread and have a high incidence (Evans 2000) in its native range, in addition to being host specific. *Endophyllum osteospermi* has been shown to be damaging to its host plant *C. monilifera* ssp. *monilifera* (Wood 2002) and is widespread in its native range (pers. obs.). However, it has been observed to occur with a high incidence in only a limited number of host plant populations (pers. obs.). Certain biocontrol agents which have limited incidences in their native range have subsequently had little success in controlling their target weed, such as *P. punctiformis* on *C. arvensis* (Frantzen 1994). This raised a concern as to the potential of *E. osteospermi* as a biocontrol agent in Australia.

The increase in number of witches' brooms on individual host plants, the positive simple infection rates, and the within host plant population increase in both incidence and severity at all sites indicated that *E. osteospermi* undergoes epidemic increase under favourable environmental conditions. The generally low incidences of *E. osteospermi* under natural conditions in South Africa may therefore be due to various historical and climatic factors. The relatively frequent fires (usually at 5–15-year intervals) that sweep through the areas where *C. monilifera* occurs (van Wilgen et al. 1992) may be particularly important as they would cause local extinction of the rust after each fire. Populations of *C. monilifera* ssp. *monilifera* and ssp. *pisifera* in the Western Cape Province seldom exceed 15 years in age (Scott 1996). The Scarborough site had been burnt approximately 5 years before this study was initiated. Despite the rapid increase in incidence of witches' brooms at the Scarborough site between 1992 and 1993, the severity remained fairly low in terms of both number of witches' brooms and infection level. This suggests that the rust had just begun to establish at this site. This population was subsequently decimated by an outbreak of a tortricid moth, preventing follow-up assessments of the development of this epidemic. It does, however, indicate the speed with which this rust fungus can become established within populations of the host plant during periods of favourable environmental conditions. Rainfall over the known distribution of *E. osteospermi* is variable, both in terms of total precipitation and variation between years (Schulze 1997). Localities where a high incidence of *E. osteospermi* have been observed have a minimum of 40 mm average rainfall per month in at least 1 month of the year, though most had more than 80 mm. However, much of the area within the distribution of *E. osteospermi* has less than 40 mm average rainfall per month over the year (Wood et al. in press). In these low rainfall areas, the rust generally occurred at low incidence or was absent (pers. obs.). These two factors may well be the most important factors contributing to the observed limited number of host plant populations with a high incidence of *E. osteospermi* in South Africa.

Morris (1982) observed that host bushes with heavy infection levels of *E. osteospermi* frequently died. A number of heavily infected bushes died during the course of this study. These observations strongly suggest that high disease incidence is associated with a greater host mortality. The actual cause of death of the plant may be a greater susceptibility to drought (Neser & Morris 1984), or invasion by secondary pathogens (Cother et al. 1996; Cother 2002).

*Endophyllum osteospermi* is capable of epidemic increase under suitable climatic conditions. It also reduces the growth and reproductive output, and may lead to increased mortality rates, of its host plant. It therefore has potential as a biological

control agent against *C. monilifera* ssp. *monilifera* in Australia. Data presented here provides a baseline for a comparison with the performance of *E. osteospermi* if it is released as a biocontrol agent. Such a comparison between incidence of an agent in its native and introduced range may assist in selection of other candidate agents that are ultimately successful.

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