CSIRO PUBLISHING

www.publish.csiro.au/journals/app *Australasian Plant Pathology*, 2004, **33**, 549–558

Predicting the distribution of *Endophyllum osteospermi* (Uredinales, Pucciniaceae) in Australia based on its climatic requirements and distribution in South Africa

A. R. Wood[A,B,D], *P. W. Crous*[B,C] *and C. L. Lennox*[A]

[A]ARC — Plant Protection Research Institute, P. Bag X5017, Stellenbosch, 7599, South Africa.
[B]Department of Plant Pathology, University of Stellenbosch, P. Bag X1, Stellenbosch, 7602, South Africa.
[C]Centraalbureau voor Schimmelcultures, PO Box 85167, 3508 AD Utrecht, The Netherlands.
[D]Corresponding author. Email: WoodA@arc.agric.za

Abstract. The perennial bush *Chrysanthemoides monilifera* ssp. *monilifera* (Asteraceae) is infected by the autoecious, microcyclic rust fungus *Endophyllum osteospermi.* Both organisms are native to South Africa, whilst the plant has also become naturalised in Australia where it is the target of a biological control program. *E. osteospermi* is under consideration as a biocontrol agent for this weed. Temperature and light requirements for aecidioid teliospore germination and basidiospore development were studied, as was the nuclear cycle during germination. Aecidioid teliospores germinated between 10 and 20°C, with 15°C as optimum temperature. Light, and particularly near-UV light, stimulated germination whereas germination was poor under dark conditions. A period of 6–8 h of light was the minimum needed to obtain germination levels equivalent to continuous light. The temperature requirements for basidiospore development differed from that for aecidioid teliospore germination. Optimal basidiospore production was at 15°C, but a rapid decrease occurred at higher temperatures, with few developing at 19°C, despite a high germination rate at this temperature. Two nuclear divisions occurred within 12 h of germination initiation to produce a metabasidium with three or four nuclei. A third nuclear division occurred in the basidiospores that then germinated between 24 and 48 h. Plants inoculated under controlled conditions took 5 to 24 months after inoculation for witches' broom symptoms to begin to develop. The detailed life cycle of *E. osteospermi* is presented. A Geographic Information System (GIS) approach was used to develop a model of the potential distribution of *E. osteospermi* in South Africa. This was based on monthly average climate surfaces with parameters derived from the above experiments. The parameters were modified so that the majority of all recorded localities of *E. osteospermi* in South Africa were included, whilst at the same time including only the minimum geographic area. The same model was applied to Australia to suggest a potential distribution of the rust fungus if released in Australia for the biological control of *C. monilifera* ssp. *monilifera*. This potential distribution was similar to one generated using the climate matching computer program CLIMEX, but gave greater spatial accuracy, at least in South Africa. Both approaches indicate that *E. osteospermi* should establish in temperate south-eastern Australia where *C. monilifera* ssp. *monilifera* is an invasive weed.

Additional keywords: nuclear cycle, life cycle, Boneseed, biological weed control.

Introduction

The perennial bush *Chrysanthemoides monilifera* ssp. *monilifera* (Asteraceae: Calendulae) is native to the winter rainfall region of the Western Cape Province of South Africa. This plant is susceptible to an autoecious, microcyclic (endocyclic) rust fungus which is native to South Africa and which produces localised systemic infections causing witches' brooms (Morris 1982). A reduction in growth and seed production of naturally infected host bushes is associated with the presence of witches' brooms (Wood 2002) and mortality of host bushes is associated with high levels of infection (Neser and Morris 1984; Wood and Crous 2005). Pycnia and aecidioid telia develop predominantly on abaxial leaf surfaces and stems, and less commonly on adaxial leaf surfaces (Morris 1982). Following germination of aecidioid teliospores, septa develop to produce three- to four-celled metabasidia (promycelia). Subsequently two to three, and only rarely four, vesicle-like structures develop from these

 10.1071/AP04064 0815-3191/04/040549

cells, which are separated by septa from the metabasidium (Morris 1982; Wood 1998). It has been postulated that these structures represent modified basidiospores (Gardner 1988; Chen *et al.* 1996). The modified basidiospores are not dispersive structures and do not separate from the metabasidia (Morris 1982; Wood 1998). The production of basidiospores by what are morphologically aeciospores is typical of an endocyclic rust fungus. Originally, this fungus was described as *Aecidium osteospermi* (Doidge 1927), but because of its endocyclic nature, it was transferred to *Endophyllum* as *E. osteospermi* (Wood 1998).

C. monilifera ssp. *monilifera* is naturalised in south-eastern Australia. It is the target of a biological control program, for which *E. osteospermi* is considered to be a suitable candidate organism (Scott and Adair 1995; Adair and Edwards 1996). Morris (1982) determined the effect of temperature on aecidioid teliospore germination, and also the nuclear cycle during germination. The work reported here was undertaken to better elucidate the effect of environmental conditions on spore germination and the development of basidiospores, as well as to verify the nuclear cycle of *E. osteospermi*.

The climate matching computer program CLIMEX (CSIRO, Australia) (Sutherst *et al*. 1999) has been used to predict the potential distribution of both weeds (e.g. McFadyen and Skarratt 1996; Holt and Boose 2000) and biological control agents (e.g. Scott 1992). A model was developed using CLIMEX, based on the epidemiological parameters determined in this study that corresponded as closely as possible with the actual distribution of *E. osteospermi* in South Africa. This same model was then used to determine the potential distribution of *E. osteospermi* in Australia.

There are rapid changes in climatic variables over short geographical distances in South Africa within the distribution range of *E. osteospermi* due to rapid altitude changes over the Cape Fold Mountains and the Drakensberg. There was a limited correspondence between the most accurate prediction of the CLIMEX model and the actual distribution of *E. osteospermi* in South Africa, because of these rapid changes in climate variables. Therefore a Geographic Information System (GIS) approach, using the computer program ArcView (Environmental Systems Research Institute, USA) was used to develop a model giving a more spatially explicit prediction of the potential distribution in South Africa, and this model was again applied to determine the potential distribution of *E. osteospermi* in Australia.

Methods

Collection of inoculum

Naturally infected plant material was collected from a site on the Cape Peninsula (2 km south of Simon's Town, 34°13′S, 18°28′E), and placed in plastic bags. This material was stored at 5°C for 4–7 days after collection. Dry aecidioid teliospores were obtained from this material by means of a homemade collection device, made by placing a small piece of filter paper over the end of a cut-down 2 mL plastic syringe, and placing this in one end of a 10-mm-diameter plastic pipe attached to an air pump. The spores were stored in plastic screw-top vials at 5°C until used.

Effect of temperature and light on aecidioid teliospore germination and basidiospore development

Dry aecidioid teliospores were sprinkled over the surface of water agar (1.5% agar, Biolab, Midrand, South Africa) in 6-cm-diameter Petri dishes and incubated for 24 h in an incubator with fluorescent white and near-UV light (13.63 μmol/s/m^2), except where otherwise indicated. Three Petri dishes were used per treatment. The following experiments were carried out: (1) germination at 10, 15, 19 and 25°C under continuous light conditions; (2) germination at 15°C under continuous light or dark conditions; (3) germination at 15°C under 24 h dark, 1 h light /23 h dark, 2 h light/22 h dark, 3 h light/21 h dark, 4 h light/20 h dark, 6 h light/18 h dark, 8 h light/16 h dark and 24 h light conditions; and (4) germination at 15°C under continuous white or near-UV light only. One hundred randomly chosen spores were observed per Petri dish and the average percentage germination determined for each treatment in each experiment. Each of these four experiments was repeated three times. Except in experiment 4, both white and near-UV fluorescent lights were used in the incubators. For germination in the dark, the Petri dishes were placed in a cardboard box wrapped in a black plastic bag in the same incubator used for germination in the light.

Dry aecidioid teliospores were sprinkled over the surface of water agar and incubated as above, at 15, 17 or 19°C. After 48 h, 100 randomly chosen germinated spores per Petri dish were observed and the average percentage with basidiospores determined for each temperature. This experiment was repeated three times.

Longevity of aecidioid teliospores, and their viability when stored at different temperatures

A sample of aecidioid teliospores was stored at 5°C for a 2-month period. At weekly intervals, dry aecidioid teliospores were dusted over the surface of water agar in three 6-cm-diameter Petri dishes and incubated as above. The average percentage germination for each week was determined after observing 100 randomly chosen spores per Petri dish. This experiment was repeated once.

A sample of aecidioid teliospores was divided into four subsamples that were stored at 5, 15, 20 or 25°C. At weekly intervals (for 1 month), spores from each temperature were plated on water agar and incubated as above. One hundred spores from each Petri dish were observed and the average percent germination was calculated for each sub-sample stored at the respective temperatures. This experiment was repeated once.

Nuclear staining of germinating aecidioid teliospores

Dry aecidioid teliospores were sprinkled on water drops on glass microscope slides and placed on moist pieces of tissue paper in 9-cm-diameter Petri dishes, which were sealed and incubated at 15°C with fluorescent white and near-UV light as above. They were incubated for 2, 3, 4, 6, 9, 12, 24 or 48 h. The germinated spores were heat-fixed onto the slides after their respective incubation time, and then immersed in Carnoy's Solution overnight (25% acetic acid, 75% ethanol). The germinated spores were then hydrated and acidified (10 min immersion sequentially in each of 96% ethanol, 70% ethanol, distilled water, cold 1 M HCl, hot (60°C) 1 M HCl, and then rinsed several times in distilled water). They were then immersed for 10 min in freshly prepared phosphate buffer solution (55 parts of a stock aqueous solution of $Na_2HPO_4.2H_2O$ (11.87 g/L) mixed with 45 parts of a stock aqueous solution of KH_2PO_4 (9.07 g/L)), following which they were stained until the nuclei were well differentiated by adding 2 drops of Gurr's improved

R66 Giemsa's Stain (Gurr, BDH Chemicals Ltd, Poole, England) per mL of phosphate buffer solution. The spores were rinsed with buffer solution and examined using a Zeiss Axioskop (Germany) light microscope. Cross sections of mature aecidioid telia made by hand were also Giemsa-stained using the above method.

Infection of whole plants

Seedlings (5–10 cm high) of *C. monilifera* ssp. *monilifera* were collected from the field, potted in 20-cm-diameter plastic pots with a mixture (1:1:1) of top soil, river sand and compost, and kept in a shade house. They were watered every second day and fed every 2 weeks with 5 g Chemicult hydroponic nutrient solution (Chemicult Products, Camps Bay, South Africa). When they were approximately 30 to 50 cm in height they were inoculated with an aqueous aecidioid teliospore suspension by means of an airbrush, and incubated for 3 days in a dew chamber in a growth room at 10–16°C. The spore suspension was applied to all leaf and stem surfaces with particular attention paid to the immature leaves at the growing tip of all branches. Twelve plants were inoculated in 1995, and 22 were inoculated in 1996. The plants were regularly observed for the development of witches' brooms for up to 2 years after inoculation. New shoots that appeared to be witches' brooms, but which had not developed pycnia or aecidioid telia, were confirmed as infected by microscopically examining hand-made cross sections of leaves. The sections were cleared for 30 min in Carnoy's solution, stained (2 min in 0.05% aniline blue in lactophenol) and then observed using a Zeiss Axioskop light microscope for the presence of hyphae and coiled haustoria (Morris 1982).

The possible distribution of E. osteospermi *in Australia*

The distribution of *E. osteospermi* in the Western Cape, Eastern Cape and KwaZulu Natal Provinces of South Africa was determined on an *ad hoc* basis during the period 1992 to 2003 and representative herbarium specimens were deposited in the South African National Collection of Fungi, ARC-PPRI, Pretoria (PREM). A total of 93 localities was recorded.

CLIMEX (CSIRO, Australia) is a dynamic simulation model enabling the prediction of an organism's geographical range using climatic parameters (Sutherst *et al*. 1999). CLIMEX was used to create a model that predicted the distribution of *E. osteospermi* in South Africa, using environmental parameters as determined by the above experiments. The model was then modified until it best approximated the known distribution of *E. osteospermi* in South Africa, and then run to generate a potential distribution for Australia. The method used was described in detail by Scott (1992) and McFadyen and Skarratt (1996).

GIS allows spatially explicit presentation of data. Monthly and annual climate data are available on a 1.6 × 1.6 km grid scale for South Africa (Schulze 1997) and on a 2.5 × 2.5 km or a 25 × 25 km grid scale for Australia (Bureau of Meteorology, Melbourne, Australia). Various datasets can be combined in any combination. A theoretical GIS approach was used in which the effect of environmental conditions on aecidioid teliospore germination and basidiospore development formed the basis for creating a map using the computer program ArcView GIS 3.1 (Environmental Systems Research Institute, USA), showing an approximation of the likelihood of infection by *E. osteospermi* occurring during July in South Africa, assuming that both host plant and inoculum are present. It was assumed that the potential geographic distribution of *E. osteospermi* was equivalent to this likelihood of infection. Localities of *E. osteospermi* recorded between the Cape Peninsula and Tsitsikamma were used to verify this map.

The 'average monthly maximum temperature' and 'average monthly rainfall' surfaces for July (Schulze 1997) were first utilised. This was because the majority of recorded localities fell within the winter or all-year rainfall regions of South Africa. These surfaces were intuitively reclassified so that the maximum number of localities was incorporated. The starting point for this reclassification was based on the optimum environmental conditions determined above. The generated map was refined using the '80% probability of annual rainfall' surface (Schulze 1997), which was reclassified so that the predicted distribution incorporated as small a surface area as possible. Classes used were: (1) 'average July maximum temperature' ≤ 20°C; (2) 'average July rainfall' 21–40 mm, 41–80 mm and ≥ 81 mm; and (3) '80% probability of annual rainfall' ≥ 301 mm. The three average monthly rainfall classes were used to show differing potential for infection; the lower the rainfall, the lower the potential and the higher the rainfall, the higher the potential. These classes were fitted to where *E. osteospermi* had been observed to be either scarce or abundant for at least several years of observation at certain localities.

Subsequently, using the same classes for the relevant surfaces, maps were generated for the months of September, November and January in South Africa, and for July, September, November and January in Australia. The Australian '10 percentile of annual rainfall' surface was used as the equivalent of the South African '80% probability of annual rainfall' (Bureau of Meteorology, Melbourne). These four months were chosen as representative for the whole year.

Results

Field observations on E. osteospermi

Pycnia were produced throughout the year on young growth of witches' brooms in advance of the aecidioid telia on individual leaves, regardless of the age of the witches' broom. Production of aecidioid telia appeared to be dependent on the amount and duration of rainfall in the wet season, as growth of the witches' brooms and production of new aecidioid telia only occurred while the host was actively growing (data not presented). Host plant growth occurs during winter and spring (approximately June to November) in the winter rainfall region of South Africa. It was noted that spores collected during hot summer months, although appearing healthy, often had a low viability. Because of this, aecidioid teliospores were only collected for use during winter and spring. During the rain season, aecidioid teliospores were produced in abundance.

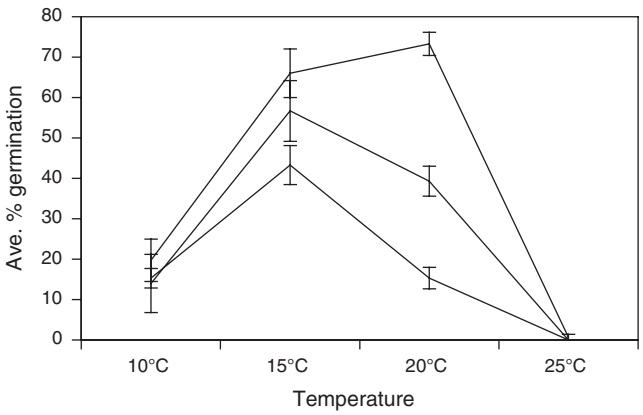


Fig. 1. Average percent germination (± standard error) of three collections of aecidioid teliospores of *Endophyllum osteospermi* at 10, 15, 20 and 25°C after 24 h of incubation under continuous light conditions.

Table 1. Percent germination of aecidioid teliospores of *Endophyllum osteospermi* under different light conditions at 15°C

Light source	% Spore germination		
	Repeat 1	Repeat 2	Repeat 3
Effect of light or dark conditions			
Dark (no light)	40.0**[A]	21.3**	3.3**
Continuous white and near-UV light	74.0	83.3	56.7
Effect of light sources			
Continuous white light only	49.3*	31.7**	24.7**
Continuous near-UV light only	83.7	81.7	58.7

[A]Averages within each repeat followed by * are significantly different ($P < 0.05$), and ** are highly significantly different ($P < 0.01$), according to one-way ANOVA. Comparisons are between dark and light conditions, and between the light sources.

Effect of temperature and light on aecidioid teliospore germination and basidiospore development

The highest percent germination of aecidioid teliospores occurred at 15°C, with 20°C having the second highest germination (Fig. 1). Light, in particular near-UV light, promoted germination (Table 1), and 6–8 h of light was the minimum period needed for optimum germination (Table 2). The optimal temperature for basidiospore production was 15°C. At 17°C, basidiospore production was much less than that at 15°C, and very few developed at 19°C (Table 3), even though there was a high percentage of aecidioid teliospores that had germinated at both 17 and 19°C.

Longevity of aecidioid teliospores, and their viability when stored at different temperatures

When stored at 5°C, the aecidioid teliospores maintained their viability for the 8 weeks tested (data not presented). Aecidioid teliospores maintained their viability for 2 weeks (after collection in the field) regardless of the temperature at which they were stored. After this, the viability of spores stored at 20 and 25°C declined rapidly, whereas those stored at 15°C declined less rapidly. Spores stored at 5°C maintained their viability over the 4 weeks of testing (Fig. 2).

Table 2. Percent germination of aecidioid teliospores of *Endophyllum osteospermi* at different durations of light and dark conditions

Light source	% Spore germination		
	Repeat 1	Repeat 2	Repeat 3
24 h dark	1.3 e[A]	25.3 e	32.0 e
1 h white and near-UV light/23 h dark	10.0 d	35.3 d	32.0 e
2 h white and near-UV light/22 h dark	14.7 d	41.0 c	36.7 d
3 h white and near-UV light/21 h dark	34.7 c	38.0 cd	54.0 b
4 h white and near-UV light/20 h dark	29.0 c	54.0 b	49.3 c
6 h white and near-UV light/18 h dark	58.3 a	53.7 b	56.3 b
8 h white and near-UV light/16 h dark	43.0 b	61.7 a	68.7 a
24 h white and near-UV light	42.7 b	64.3 a	69.7 a
LSD	7.6	4.5	4.3

[A]Averages followed by different letters are significantly different according to the LSD calculated for each repeat.

Table 3. Average percent (± standard error) of germinated aecidioid teliospores of *Endophyllum osteospermi* that produced vesicle-like modified basidiospores after 48 h incubation at different temperatures

Temperature (°C)	% Basidiospore development		
	Repeat 1	Repeat 2	Repeat 3
15	80.0 ± 3.5	84.7 ± 2.9	73.0 ± 3.2
17	24.0 ± 7.2	46.3 ± 7.3	47.3 ± 5.7
19	3.7 ± 0.3	9.3 ± 1.8	22.0 ± 3.1

Nuclear staining of germinating aecidioid teliospores

The spore mother cells within the aecidioid telium were binucleate and divided to produce two binucleate cells. The proximal cell remained binucleate, becoming the intercalary cell. The distal cell expanded and became an aecidioid teliospore, which was binucleate with two small nuclei when immature but became uninucleate with one large nucleus at maturity (Fig. 3). All mature spores observed

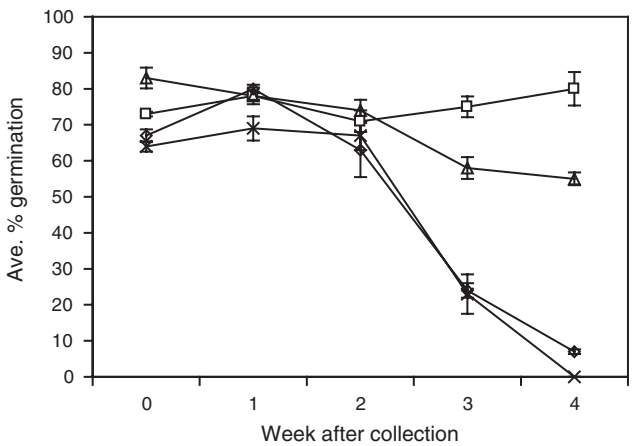
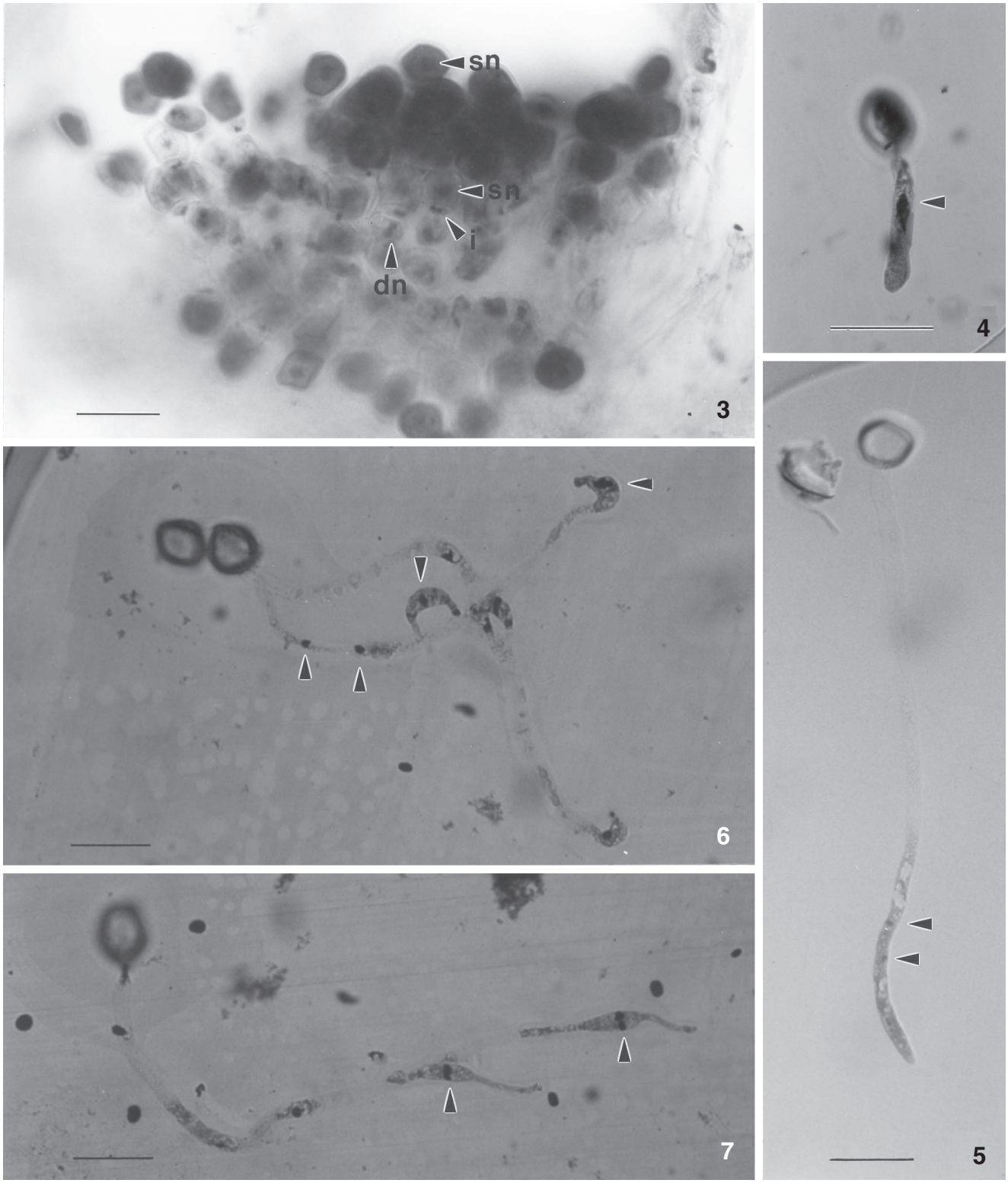


Fig. 2. Average percent germination (± standard error) of a single collection of aecidioid teliospores of *Endophyllum osteospermi* stored at 5 (□), 15 (△), 20 (×) and 25°C (◇) for 4 weeks after collection. Germination was determined after 24 h incubation at 15°C under continuous light conditions.



Figs 3–7. Aecidioid telia and germinating aecidioid teliospores of *Endophyllum osteospermi*. **Fig. 3.** Transverse cross section through an aecidioid telium with stained nuclei, immature spores (dn) and intercalary cells (i) have two nuclei whereas mature spores (sn) have a single nucleus. **Fig. 4.** Showing single nucleus (arrow) that has moved from inside the aecidioid teliospore into the germ tube, at 3 h after the onset of germination. **Fig. 5.** Showing two nuclei (arrows) in the germ tube, at 6 h after the onset of germination. **Fig. 6.** Showing four nuclei (arrows) in the metabasidium of which the distal two have moved into vesicle-like modified basidiospores, at 12 h after the onset of germination. **Fig. 7.** Showing two nuclei in each of the vesicle-like modified basidiospores (arrows) which have germinated, at 24–48 h after the onset of germination. Scale bar = 40 µm.

were uninucleate. During spore germination the following sequence of events occurred: (1) after 3 h of incubation the single nucleus had moved out of the spore into the growing metabasidium (Fig. 4); (2) after 6 h, two nuclei were present in the metabasidium after a first nuclear division (Fig. 5); (3) after 9 h, a first septum had been produced in the metabasidium and four nuclei were present after a second nuclear division; (4) after 12 h, one or two more septa had been produced dividing the metabasidium into three or four cells, and two, three or four vesicle-like modified basidiospores had developed, and single nuclei had migrated into each of the basidiospores (Fig. 6); and (5) after 24 h, two nuclei were usually present in at least one basidiospore and after 48 h, all basidiospores had two nuclei after a third nuclear division (Fig. 7).

Infection of whole plants

Of the 12 plants inoculated in 1995, four developed witches' brooms during 1996 and a further two developed them during 1997. Of the 22 plants inoculated in 1996, four developed witches' brooms in 1997 and a further ten developed them during 1998. Of these witches' brooms, the first began to develop on a plant 5 months after inoculation. The last witches' broom began to develop 24 months after inoculation. Systemic infection of all witches' brooms was confirmed either by the presence of pycnia and aecidioid telia, or by microscopic examination of leaf cross sections to detect the intercellular hyphae and tightly coiled intracellular haustoria produced by this rust fungus.

The possible distribution of E. osteospermi *in Australia*

Temperature and rainfall were the most important climate parameters in the model generated using CLIMEX. The distribution predicted in South Africa corresponded to higher rainfall areas of the temperate region (with both winter and all-year rainfall seasons) in South Africa (Fig. 8).

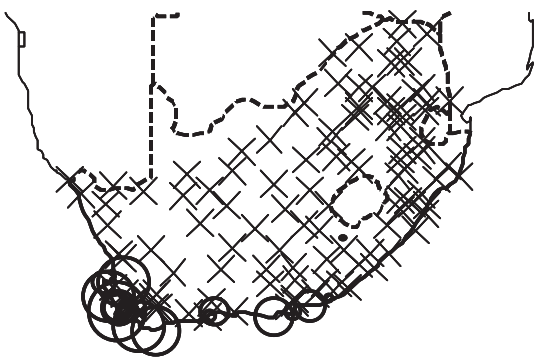


Fig. 8. Map of South Africa, generated by CLIMEX, showing locations suitable for infection by *Endophyllum osteospermi* to occur. Circles indicate a suitable location (the larger the circle the greater the suitability), and crosses indicate unsuitable localities. Each location represents a weather station at the epicentre of the circles or crosses.

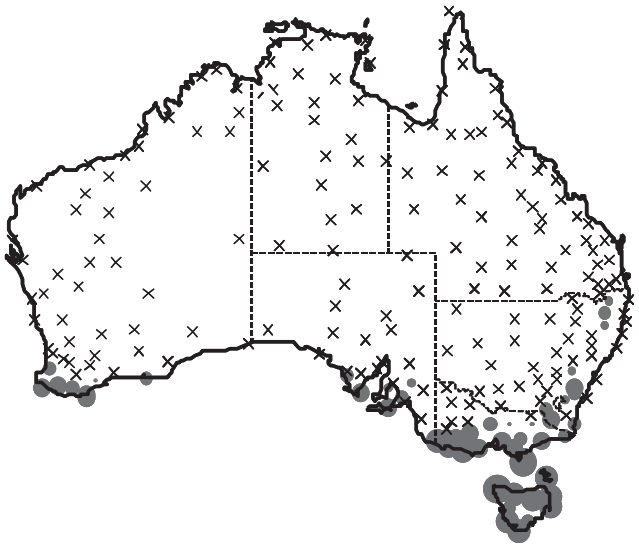


Fig. 9. Map of Australia, generated by CLIMEX, showing locations suitable for infection by *Endophyllum osteospermi* to occur. Circles indicate a suitable location (the larger the circle the greater the suitability), and crosses indicate unsuitable localities. Each location represents a weather station at the epicentre of the circles or crosses.

The predicted Australian distribution corresponded with the temperate region of Australia (Stern *et al*. 2003) (Fig. 9).

The predicted distributions of *E. osteospermi* generated with the GIS approach for the months of July, September, November and January for both South Africa (Fig. 10) and Australia (Fig. 11) are presented. A total of 75 localities fell within the area of possible distribution for the month of July, and only 18 outside. Most of these latter localities were in the summer rainfall region. The predicted distribution of *E. osteospermi* generated with CLIMEX was similar to the above for Australia, but was more restricted in South Africa.

Discussion

Morris (1982) provided details on the macroscopic and microscopic symptoms of infection by *E. osteospermi* on its host plant *C. monilifera* ssp. *monilifera*, as well as the effect of temperature on germination of its spores and its nuclear cycle. The work reported here was carried out to verify and expand on this existing knowledge, as a preliminary step to developing a reliable method of inoculation for host specificity testing of *E. osteospermi*. In a few respects conclusions drawn from the work reported on here differ from those of Morris (1982).

Morris (1982) stated that pycnia production occurred only at the beginning of the growth season, occurring only during March and April, and preceded the development of any aecidioid telia on whole witches' brooms. This coincides with the onset of the winter rains in the south-western Cape.

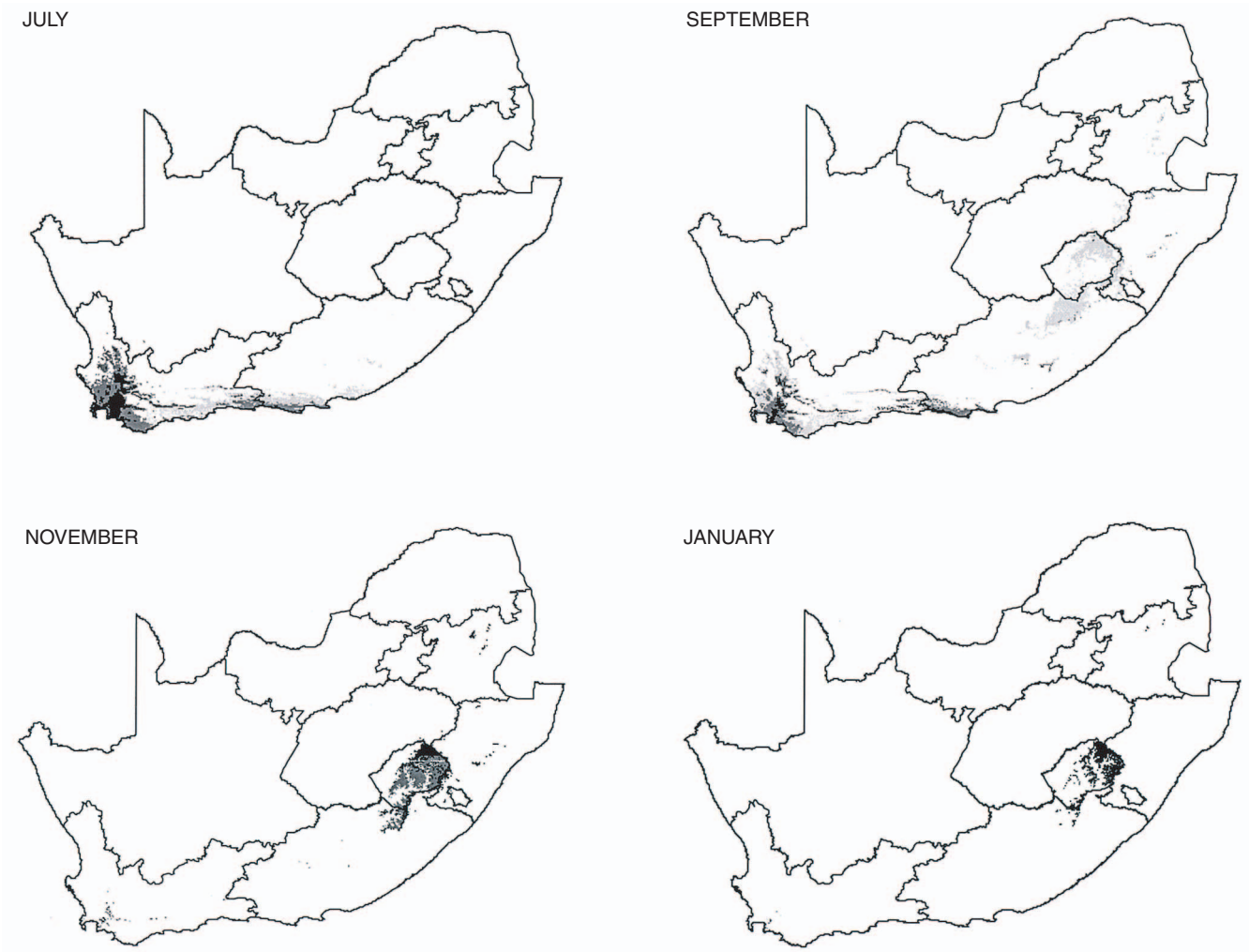


Fig. 10. Approximation of the potential of *Endophyllum osteospermi* to infect suitable host plants in South Africa for the months of July, September, November and January. Light grey = low potential, medium grey = medium potential, black = high potential, white = unsuitable climate.

It was observed, however, that pycnia production occurred throughout the growth season, preceding development of aecidioid telia on each newly produced leaf, regardless of when these grew.

Morris (1982) reported 16°C to be the optimal temperature for germination, with good germination also at 12 and 20°C. This was verified in this study. However, he recorded germination after only 6 h and, therefore, did not observe that basidiospore development had a different temperature requirement to aecidioid teliospore germination. Thus, despite good germination at 19–20°C, infection is unlikely to occur at these temperatures as few basidiospores develop. Considering the lower levels of germination at 10–12°C, there is a narrow range around 15°C of optimal temperature conditions for infection of the host plant to occur. This is discussed further below.

The nuclear cycle was essentially as reported by Morris (1982), except that he stated that typically the modified basidiospores were uninucleate except for the occasional binucleate one due to two nuclei migrating into it. Unfortunately, no times of these observations were given. It was found in this study that the basidiospores always became binucleate by means of a nuclear division, this nuclear division taking place between 24 and 48 h. Rust fungi basidiospores are commonly binucleate (Gold and Mendgen 1991). It was observed that often the nucleus in the proximal cell to the aecidioid teliospore did not undergo a second nucleus division, producing a metabasidium with only three nuclei. In all such cases, only two basidiospores were produced.

Field observations indicated that witches' brooms from branches produced during a single year's growth developed only at the end of that growth season, whereas on branches produced during the previous 1 or 2 years growth witches' brooms would develop even at the beginning of the growth season. This indicated that initiation of a witches' broom

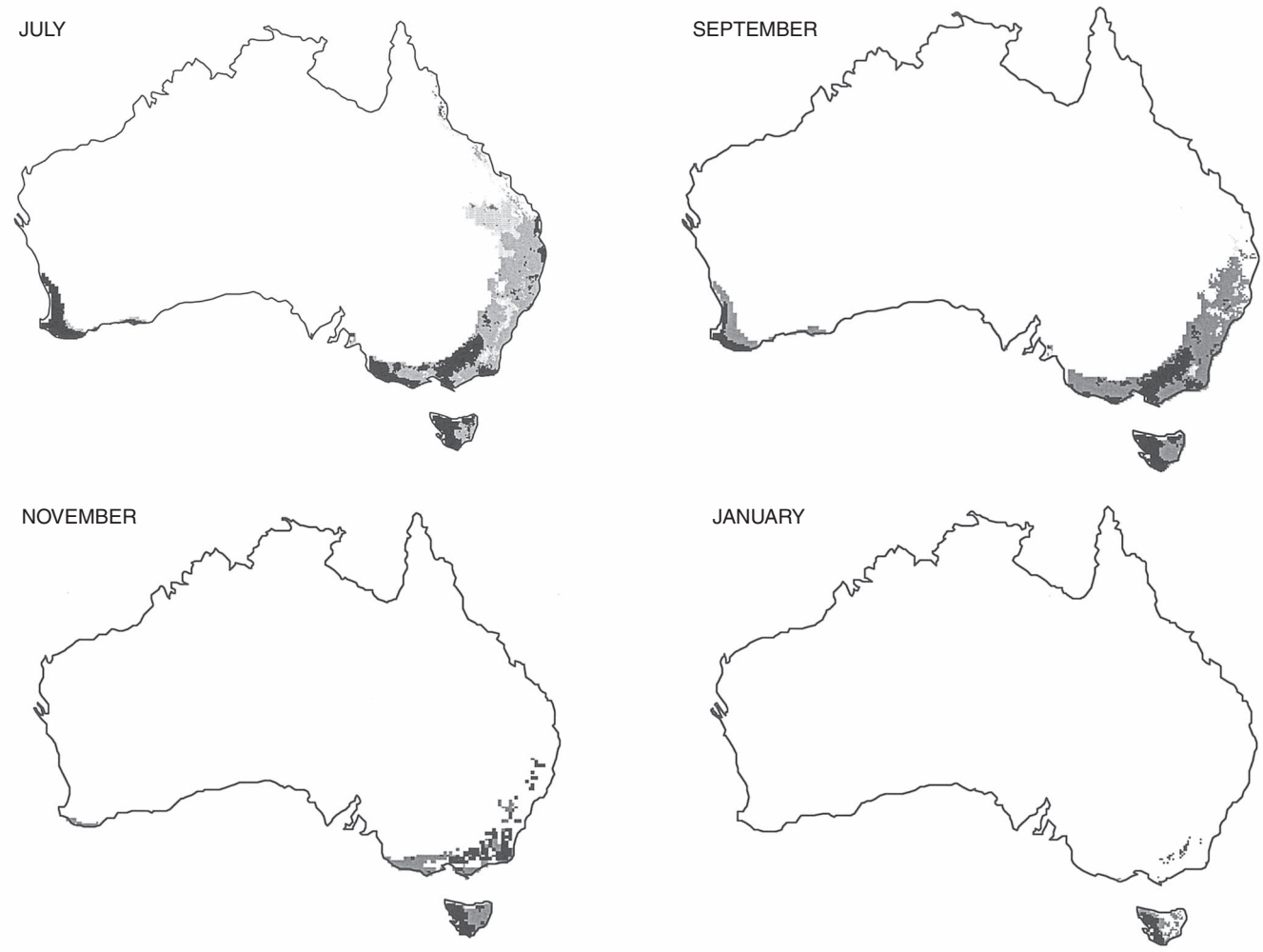


Fig. 11. Approximation of the potential of *Endophyllum osteospermi* to infect suitable host plants in Australia for the months of July, September, November and January. Light grey = low potential, medium grey = medium potential, black = high potential, white = unsuitable climate.

took from several months to over 2 years from the time at which infection occurred. This was verified by the whole plant inoculations in which witches' brooms took between 5 and 24 months to begin to develop. Field observations indicate that, once developed, individual witches' brooms survive from several months to a few years. The detailed life cycle of *E. osteospermi* has, therefore, been elucidated and is shown in Fig. 12.

E. osteospermi is well adapted to a temperate climate with seasonal rainfall. The production of localised systemic infections (witches' brooms) on a perennial, non-senescing, woody host allows for over-summering (during warm to hot dry summers) or over-wintering (during cool dry winters) without the need for producing the typical rust fungus dormant spores (teliospores). This habit was essential in allowing the contraction of the life cycle to an endocyclic one. Microcyclic life cycles commonly occur in rust fungi as an adaptation to harsh environments, such as desert (Anikster and Wahl 1979), arctic (Savile 1953) and alpine (Savile 1964) conditions. These environments all have in common short growth seasons, as does the environment in which *E. osteospermi* and its host plant *C. monilifera* ssp. *monilifera* occur naturally in South Africa. The perennial mycelium also allows infective spores to be produced very rapidly with the onset of suitable weather. Further adaptations to a temperate climate are the light requirements for spore germination and the low temperature requirement for the production of modified basidiospores. These two adaptations in combination ensure that the spores are more likely to germinate during a rain period rather than in overnight dew. This is particularly important given the length of time (2–3 days) necessary for penetration into the host leaf epidermis to occur (Morris 1982; Wood 1997).

The theoretical GIS exercise done here to suggest the potential distribution of *E. osteospermi* was possible because of having two datasets: firstly, laboratory studies of the effect

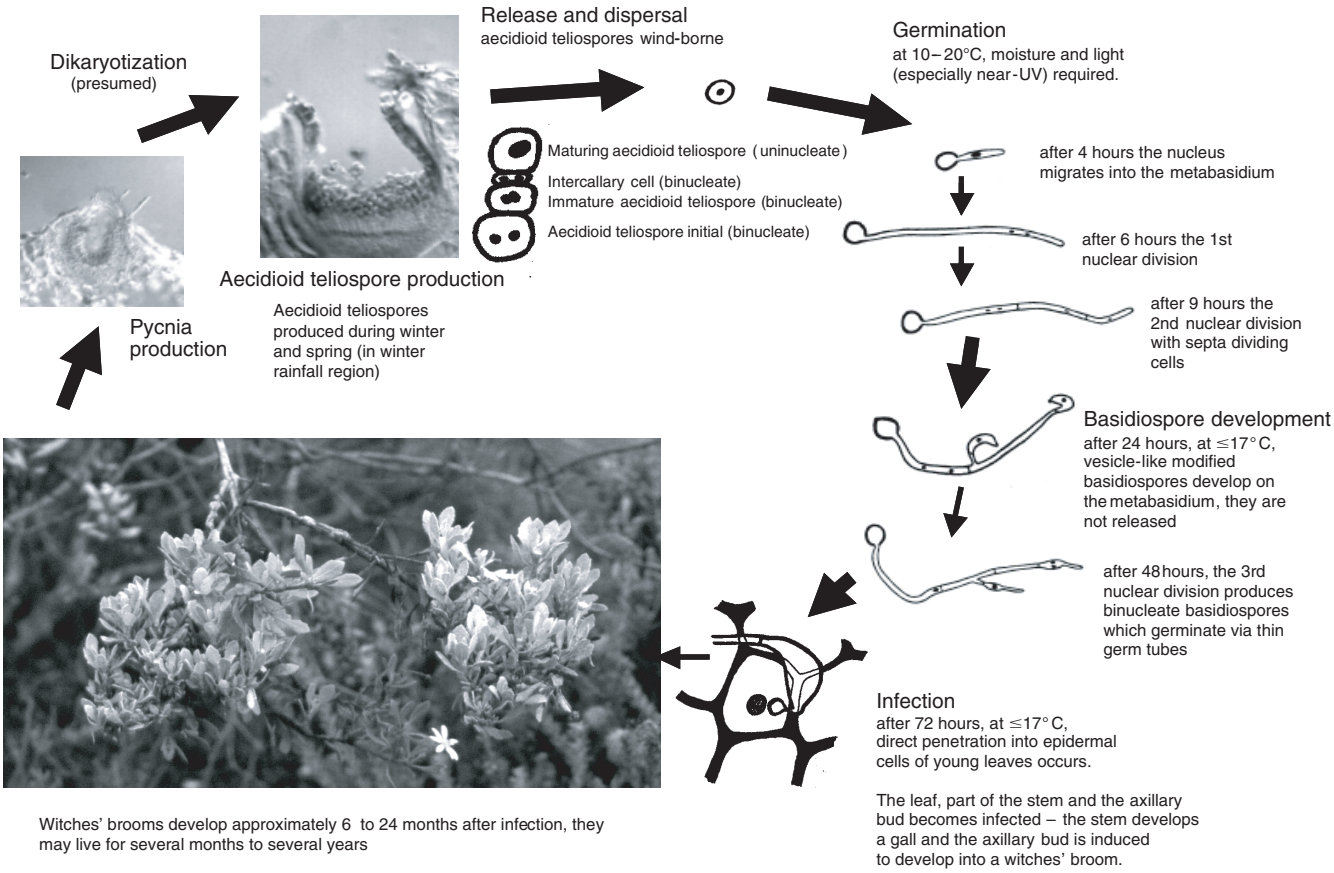


Fig. 12. Life cycle of *Endophyllum osteospermi* on its host plant *Chrysanthemoides monilifera* ssp. *monilifera*.

of environmental conditions on germination of aecidioid teliospores and production of basidiospores of *E. osteospermi* (and therefore of the climatic conditions necessary for infection), and secondly, also details on the distribution of this rust fungus in South Africa. A simple GIS model was created that gave a good approximation of the actual distribution in South Africa. The suggested distribution of this rust fungus is equivalent to the likelihood of infection, given that a suitable host plant is present, and the greater the number of months in which a specific locality has a likelihood of infection, then the greater the chance of the rust being present and persistent at that locality. Also the higher the rainfall, the greater the likelihood of infection occurring. The possible distribution of *E. osteospermi* does not necessarily coincide with the distribution of its host plant in South Africa. The potential distribution includes some areas where the plant is absent, and a large portion of the distribution of *Chrysanthemoides* is either unsuitable or marginal for *E. osteospermi*.

Based on the optimal environmental conditions determined by this study, and verified against the actual distribution of *E. osteospermi* in South Africa, the potential distribution of this rust fungus in Australia has been postulated. This area corresponds to the 'no dry season (warm summer)' and 'no dry season (mild summer)' subdivisions of the Temperate climatic region of Australia (Stern *et al*. 2003). The potential distribution (of July only) extended into a limited part of the Tropical climate region of Australia, an unexpected result. This may be due to differences in climate between South Africa and Australia, in particular, the high annual rainfall which occurs throughout the year in parts of the Tropical climate region of Australia. No area in South Africa has a comparable climate and, therefore, was not a factor in developing the model. It is unlikely that *E. osteospermi* would actually establish in these tropical areas, due to unfavourable temperatures. In changing time scales by using average monthly climate data to approximate daily climate data, a degree of error would have been introduced. Interpretation of the results of such an exercise as done above should always be done with caution, but should not detract from its usefulness.

Though largely agreeing with the GIS approach used here, CLIMEX also indicated that the 'distinctly dry (and mild) summer' subdivision of the Temperate climatic region of Australia (Stern *et al*. 2003) is suitable for infection to occur. This rust fungus, like its host plant *C. monilifera*

ssp. *monilifera*, is likely to establish in temperate south-eastern Australia if introduced as a biological control agent. It is interesting to note that apparent ideal environmental conditions for *E. osteospermi* occur over a much larger geographical area in Australia than in South Africa. Another difference is that, in Australia, the potential for infection shrinks during the year going from winter (July) to summer (January), whereas in South Africa there is a shift eastwards between winter and summer rainfall areas. Although infection will possibly occur in a much wider area, the impact of this rust fungus on the weed would be greatest in Tasmania and southern Victoria, which are apparently climatically suitable for the development of this rust fungus, according to both CLIMEX and the GIS approach used. *E. osteospermi* may, therefore, exert considerable control on *C. monilifera* ssp. *monilifera* in these regions.

Acknowledgements

ANZECC (Australian and New Zealand Environment and Conservation Council) is gratefully acknowledged for financial assistance. The Australian Bureau of Meteorology is gratefully acknowledged for providing the Australian climate surfaces. Graham de Hoedt and Shivanthi Chitty were particularly helpful. Bertie van der Merwe (ARC-Institute for Soil, Climate and Water, Stellenbosch) is thanked for help with the GIS manipulation. The technical assistance of Gwen A. Samuels (ARC-PPRI) is gratefully acknowledged.

References

Adair RJ, Edwards PB (1996) An attack strategy against *Chrysanthemoides monilifera*, a weed of native vegetation in Australia. In 'Proceedings of the IX international symposium on biological control of weeds'. (Eds VC Moran, JH Hoffmann) pp. 429–434. (University of Cape Town: Cape Town)

Anikster Y, Wahl I (1979) Coevolution of the rust fungi on Gramineae and Liliaceae and their hosts. *Annual Review of Phytopathology* **17**, 367–403. doi: 10.1146/ANNUREV.PY.17.090179.002055

Chen W-Q, Gardner DE, Webb DT (1996) Biology and life cycle of *Atelocauda koae*, an unusual demicyclic rust. *Mycoscience* **37**, 91–98.

Doidge EM (1927) A preliminary study of the South African rust fungi. *Bothalia* **2**, 1–228.

Gardner DE (1988) Teliospore germination of *Puccinia vitata*, an endemic Hawaiian rust. *Mycologia* **80**, 590–593.

Gold RE, Mendgen K (1991) Rust basidiospore germlings and disease initiation. In 'The fungal spore and disease initiation in plants and animals'. (Eds GT Cole, HC Hoch) pp. 67–99. (Plenum Press: New York, NY)

Holt JS, Boose AB (2000) Potential for spread of *Abutilon theophrasti* in California. *Weed Science* **48**, 43–52.

McFadyen RC, Skarratt B (1996) Potential distribution of *Chromolaena odorata* (siam weed) in Australia, Africa and Oceania. *Agriculture Ecosystems and Environment* **59**, 89–96. doi: 10.1016/0167-8809(96)01035-3

Morris MJ (1982) A systemic rust fungus infecting *Chrysanthemoides monilifera* ssp. *monilifera* in South Africa. *Phytophylactica* **14**, 31–34.

Neser S, Morris MJ (1984) Preliminary observations on natural enemies of *Chrysanthemoides monilifera* in South Africa. In 'Bitou bush and boneseed: a national conference on *Chrysanthemoides monilifera*'. (Eds A Love, R Dyason) pp. 105–109. (NSW National Parks and Wildlife Services and NSW Department of Agriculture)

Savile DBO (1953) Short-season adaptations in the rust fungi. *Mycologia* **45**, 75–87.

Savile DBO (1964) Geographic variation and gene flow in *Puccinia cruciferarum. Mycologia* **56**, 241–248.

Schulze RE (1997) South African atlas of agrohydrology and -climatology. Report No. TT82/96, Water Research Commission, Pretoria. (Electronic compilation by Horan M, Lynch SD, Knoesen DM, King KN, Zulu JT)

Scott JK (1992) Biology and climatic requirements of *Perapion antiquum* (Coleoptera: Apionidae) in southern Africa: implications for the biological control of *Emex* spp. in Australia. *Bulletin of Entomological Research* **82**, 399–406.

Scott JK, Adair RJ (1995) Selection of biological control agents for *Chrysanthemoides monilifera*, a weed of protected natural areas in Australia. In 'Proceedings of the 8th international symposium on biological control of weeds'. (Eds ES Delfosse, RR Scott) pp. 61–67. [(DSIR/CSIRO: Melbourne)]

Stern H, de Hoedt G, Ernst J (2003) Objective classification of Australian climates. Available online at: http://www.bom.gov.au/climate/environ/other/koppen_explain.shtml and http://www.bom.gov.au/climate/environ/other/kpn.jpg (accessed 23 September 2003).

Sutherst RW, Maywald GF, Yonow T, Stevens PM (1999) 'CLIMEX©, predicting the effects of climate on plants and animals'. (CSIRO Publishing: Collingwood, Vic.)

Wood AR (1997) Infection of the Daisy Bush *Chrysanthemoides monilifera* by the rust fungus *Aecidium osteospermi. South African Journal of Science* **93**, xiv [abstract].

Wood AR (1998) *Endophyllum osteospermi*, a new combination for *Aecidium osteospermi* (Basidiomycetes–Uredinales–Pucciniaceae). *South African Journal of Botany* **64**, 146.

Wood AR (2002) Infection of *Chrysanthemoides monilifera* ssp. *monilifera* by the rust fungus *Endophyllum osteospermi* is associated with a reduction in vegetative growth and reproduction. *Australasian Plant Pathology* **31**, 409–415. doi: 10.1071/AP02054

Wood AR, Crous PW (2005) Epidemic increase of *Endophyllum osteospermi* (Uredinales, Pucciniaceae) on *Chrysanthemoides monilifera. Biocontrol Science and Technology* (in press).

Received 27 November 2003, accepted 4 June 2004