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# Information package to support the application to release the rust fungus *Puccinia cnici-oleracei* (ex. *Conyza*) for the biological control of flaxleaf fleabane (*Conyza bonariensis*) in Australia

**Louise Morin, Kylie B. Ireland[1], Caroline Delaisse, Isabel Zeil-Rolfe and Gavin C. Hunter**

CSIRO Health & Biosecurity, Canberra, ACT

[1] Present address: Centre for Crop and Disease Management, Curtin University, Perth, WA

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# Executive summary

*Conyza bonariensis* (flaxleaf fleabane; Asteraceae) was endorsed as a target for biological control in Australia in November 2017 by the Invasive Plants and Animals Committee (now the Environment and Invasives Committee).

A microcyclic rust fungus that morphologically agreed with the description of *Puccinia cnici-oleracei* was recorded on *Conyza* species during surveys of natural enemies in Colombia. The fungus was found only on plants putatively identified as *C. bonariensis* and *Conyza sumatrensis* using molecular markers developed in Australia. A cross-inoculation experiment with accessions of *P. cnici-oleracei* from a *Conyza* sp. and from *Emilia sonchifolia* revealed that the fungus was capable of only infecting the host species it originated from, indicating a high level of specialisation. Based on these results, the accession from a *Conyza* sp. used in subsequent work was referred to as *P. cnici-oleracei* (ex. *Conyza*) to clearly indicate that it originates from this genus and thus avoid possible confusion.

Leaf samples infected by *P. cnici-oleracei* (ex. *Conyza*) were imported from Colombia into the BC3 Microbiological Area (AA A1280) of the CSIRO Black Mountain Containment Facility in Canberra in November 2018. A purified culture of the fungus was established on an Australian accession of *C. bonariensis*, and a series of experiments were performed to investigate its host-range on non-target plant species. The selection of plant species for testing was based on recent molecular phylogenies of the family Asteraceae and comprised a total of 51 representative non-target species in the subfamily Asteroideae, to which *C. bonariensis* belongs, that occur in Australia. Most species were tested in at least two separate experiments using different accessions of plant material, with *C. bonariensis* plants used as positive controls in all experiments.

Results showed that *P. cnici-oleracei* (ex. *Conyza*) is highly specific to *C. bonariensis*. The fungus successfully developed and produced abundant, normal-sized telia only on the nine Australian accessions of *C. bonariensis* tested. Chlorotic and necrotic flecks were observed on the introduced species *Eschenbachia leucantha* (previously known as *Conyza leucantha*) in two experiments, and very few pin-sized telia developed in one or two replicate plants in each experiment. These pin-sized telia were used to inoculate the highly susceptible *C. bonariensis*, and infection occurred only with a single telium from *E. leucantha*. Despite this result, it is highly unlikely that *P. cnici-oleracei* (ex. *Conyza*) would be able to complete its life cycle on *E. leucantha* in the laboratory or the field considering the low level of susceptibility of this species. While some plants of *C. sumatrensis, Grindelia camporum*, *Calendula officinalis*, *Bidens pilosa* and *Adenostemma lavenia* developed a few chlorotic flecks and/or necrotic blotches, the fungus never produced any telia on these species. All other non-target plant species tested did not develop any visible symptoms and were rated as either immune or highly resistant.

Based on these results, the level of risk associated with releasing *P. cnici-oleracei* (ex. *Conyza*) in Australia is considered negligible, and permission for its release from the biosecurity containment facility is thus sought.

# 1 Information on the target species in Australia

## 1.1 Taxonomy

| | |
|---|---|
| Clade: | Asterids, eudicots |
| Order: | Asterales |
| Family: | Asteraceae |
| Subfamily: | Asteroideae |
| Tribe: | Astereae |
| Subtribe: | Conyzinae |
| Genus: | *Conyza* |
| Species: | *bonariensis* (L.) Cronquist |
| Common names: | flaxleaf fleabane, fleabane, hairy horseweed, wavy leaf fleabane, rough conyza, Argentine fleabane |
| Synonyms: | *Conyza ambigua* DC., *Conyza crispa* (Pourret) Rupr., *Erigeron bonariensis* L., *Erigeron crispus* (Pourret)., *Erigeron linifolius* wild |

It is noteworthy, that *Conyza* is either considered by taxonomists as a genus closely related to *Erigeron* or alternatively as belonging to *Erigeron* sensu lato (Nesom 2008, Noyer 2000). Despite *E. bonariensis* being the accepted name for the weed in the Australian Plant Census[1] and Atlas of Living Australia[2], we have decided to continue using the genus name *Conyza* for the introduced and naturalised species in Australia, including *C. bonariensis*, since it has been and is still widely used. Indeed, despite a recent revision for some other species in the genus, the NSW Herbarium still retains *C. bonariensis.*[3]

## 1.2 Description

*Conyza bonariensis* grows up to 1 m in height and has erect, multiple-branching stems covered with stiff hairs (Wu 2007). Leaves are grey-green, coarsely toothed and covered in fine hairs (Fig. 1a, b). A key diagnostic feature is that upper leaves tend to be oblong to linear and entire (not toothed). Branches often grow longer than the main plant axis, from which small flower-heads are borne in leafy clusters towards the tips of the racemes. The species is characterised by the production of fluffy, cream-coloured seed heads (Fig. 1c). *Conyza bonariensis* seeds are enclosed

[1] https://www.anbg.gov.au/chah/apc/, except for Western Australia and Tasmania.

[2] https://www.ala.org.au/

[3] http://plantnet.rbgsyd.nsw.gov.au/cgi-bin/NSWfl.pl?page=nswfl&search=yes&namesearch=conyza&showsyn=OK&dist=

singly in small hard achenes, to which a tuft of fine bristles called the pappus is attached (Fig. 1d). These tufts give *C. bonariensis* its characteristic fluffy seed-head appearance.

## 1.3 Native range

*Conyza bonariensis* is native to warm temperate South America, where it was first described (Michael 1977; Nesom 2008) (Fig. 2). It is considered widespread in Argentina, Uruguay, Paraguay and Brazil, and has been recorded in coffee plantations in Colombia and Venezuela (Mangolin et al. 2012).

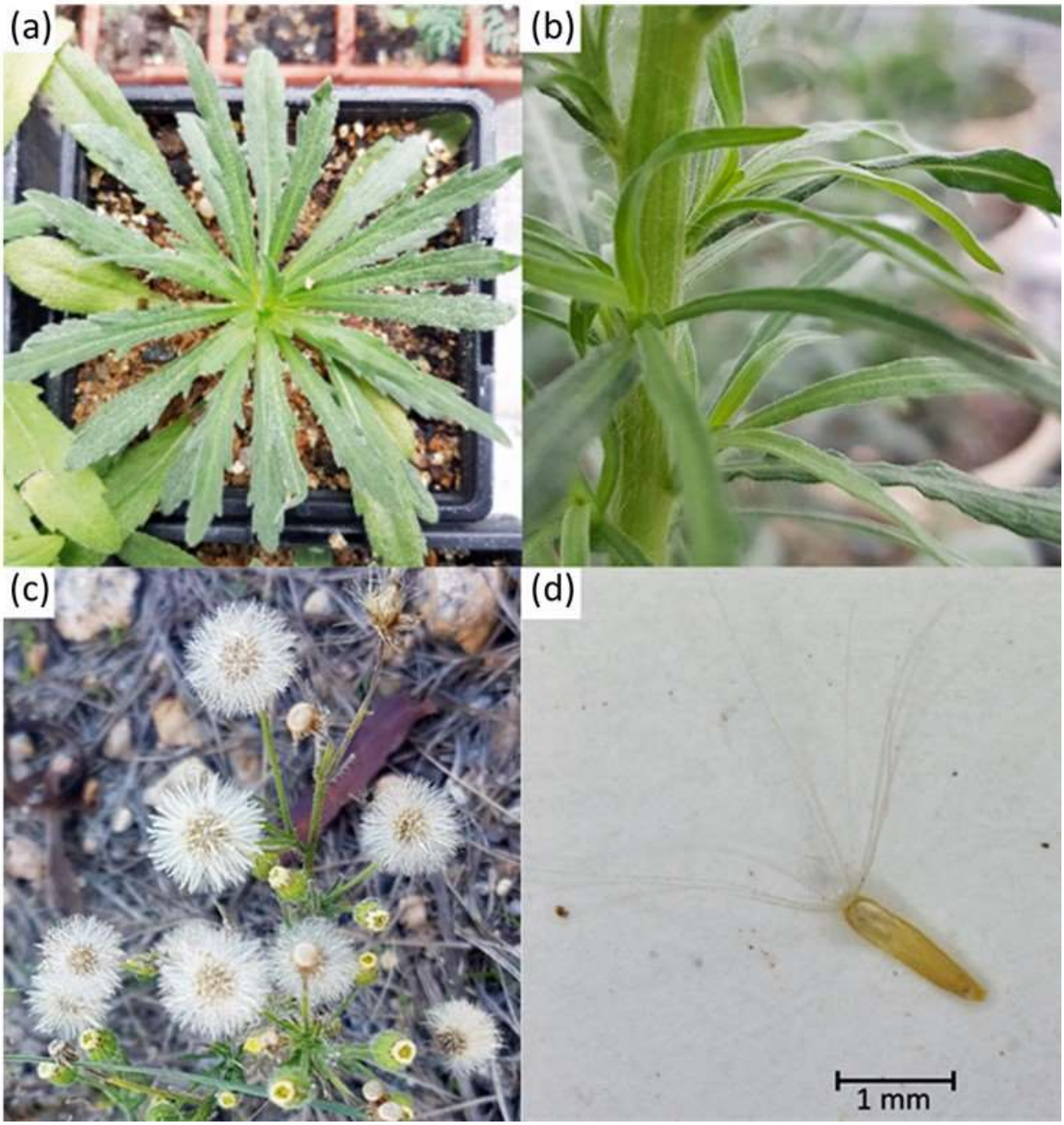


**Figure 1 Morphological features of *Conyza bonariensis*. (a) Late stage rosette prior to bolting; (b) Fine hairs covering the stems and leaves; (c) Fluffy, cream-coloured seed heads; and (d) Seed with a tuft of fine bristles. (Photos: CSIRO)**

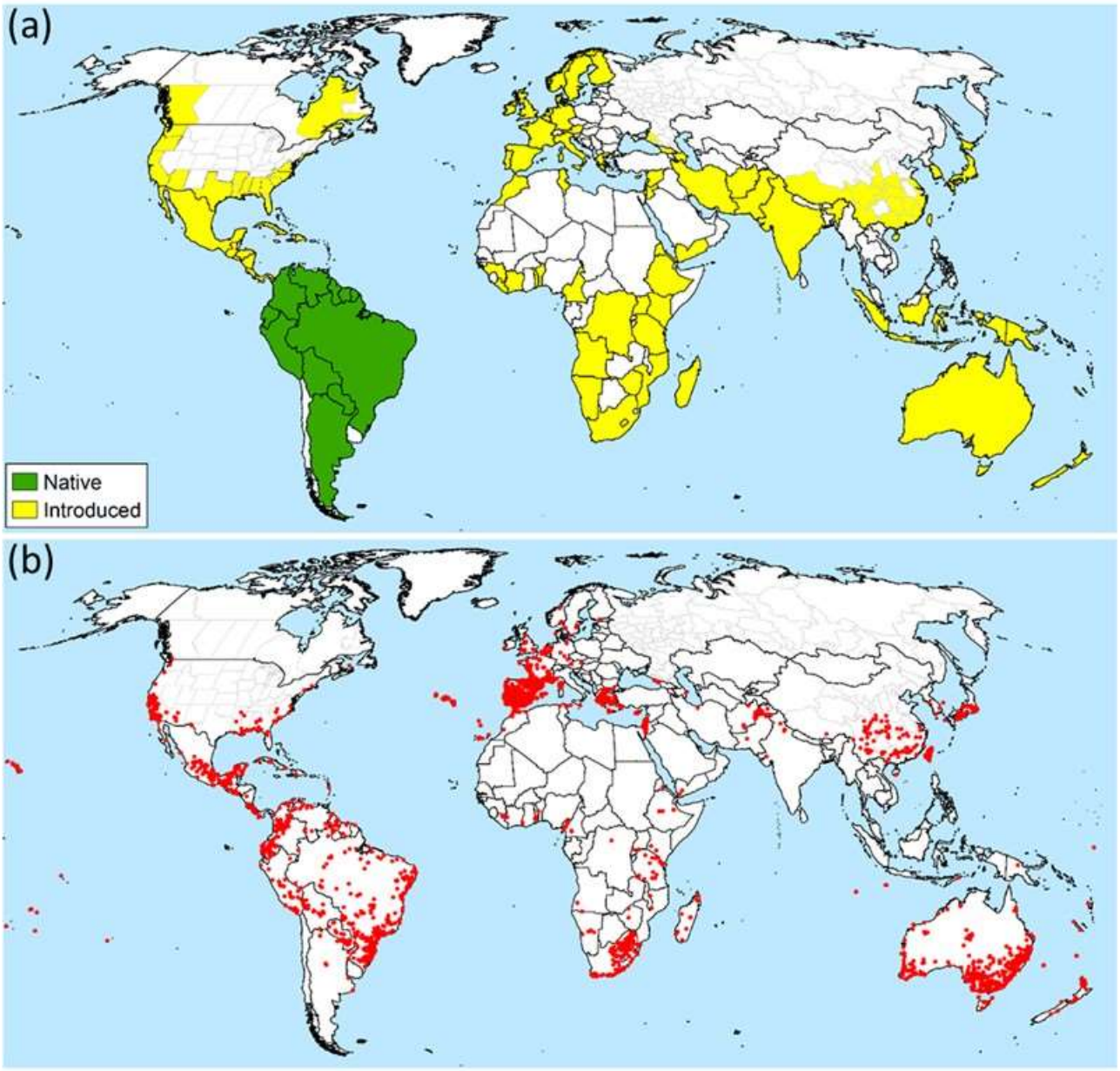


**Figure 2 Global distribution of *Conyza bonariensis*. (a) Administrative level distribution assigned to native or introduced status at the national or provincial level (when not widely distributed across a whole country), modified from Scott et al. (2016) and updated to reflect (b) point distribution data records from GBIF.org (2nd November 2018).**

## 1.4 Distribution

*Conyza bonariensis* is a cosmopolitan weed found on all continents except Antarctica (Fig. 2). It is considered as introduced to Mexico (Rios and Garcia 1998), although it is sometimes listed as native to Central America (Scott et al. 2016). Elsewhere, the plant is widespread in Europe, the Mediterranean basin, southern and eastern Africa and Australia, and is recorded as present in Asia, New Zealand and some Pacific Islands (GBIF.org 2nd November 2018; Michael 1977; Scott et al. 2016) (Fig. 2). *Conyza bonariensis* was documented as growing in European gardens in the first

half of the 18th century (Michael 1977). It may therefore have been most likely introduced to Australia from Europe and/or America (Michael 1977; Wu 2007).

*Conyza bonariensis* is present in all states of Australia, occurring predominantly in temperate and Mediterranean coastal regions, and with restricted distributions in semi-arid to arid central regions (GBIF.org 2nd November 2018; Michael 1977; Scott et al. 2016; Wu 2007) (Fig. 3a). It was mostly likely accidentally introduced into South Australia at some time in the first half of the 19th century, as it was widespread in the Adelaide area when the first botanical collections of the species were made, in 1847 (Burry and Kloot 1982; GBIF.org 2nd November 2018; Michael 1977; Wu 2007). Significant areas of Queensland, New South Wales, South Australia, Tasmania and Western Australia have been identified as either optimal or suitable environments for growth of *C. bonariensis* (Fig. 3b), so the distribution and abundance of the weed in these states could increase (CSIRO unpublished data).

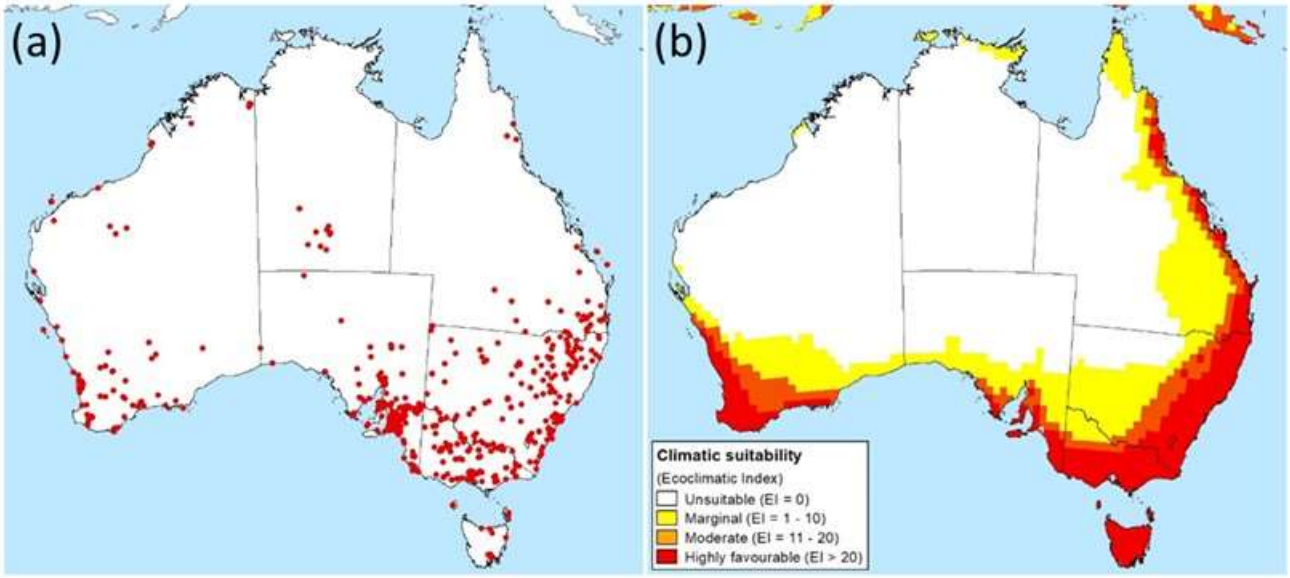


**Figure 3 *Conyza bonariensis* (a) current distribution in Australia and (b) projected climatic suitability, as modelled using CLIMEX with the CliMond dataset of 1981-2010 climate normal (CSIRO unpublished data). Increased intensity of red colour, starting from yellow, indicates higher climatic suitability.**

## 1.5 Current status in Australia, including summary of the economic and environmental losses caused by the target

*Conyza bonariensis* is a significant agricultural weed in Australia. It is a major problem in the grain growing regions of northern New South Wales and southern Queensland, with an increasing prevalence and importance to the southern and western grains regions (Widderick et al. 2011). It has been ranked as the third most problematic weed in summer fallow in Australia, where it has been estimated to affect an area of nearly 3 million ha, causing revenue losses in excess of $43 million annually (Llewellyn et al. 2016). Its impacts are expected only to increase, especially given that glyphosate-resistant populations have developed in the northern grain growing region (Walker et al. 2011). It is also a common weed of horticulture and disturbed areas such as roadsides in Perth and through much of the south-west of Western Australia (Hussey et al. 2007).

A workshop held in 2004 identified the following factors that may have favoured the emergence of fleabane, primarily referring to *C. bonariensis*, as one of the most difficult-to-control weeds in dry-land cropping systems in Australia (Walker et al. 2004):

- Greater adoption of zero tillage, as fleabane is much more of a problem in systems that do not use tillage.
- Trend towards using glyphosate alone in fallows, which seems less effective than glyphosate mixtures.
- Reduction in use of group B herbicides in wheat, particularly chlorsulfuron, as fleabane seem susceptible to these herbicides.
- Introduction of skip rows in sorghum resulting in reduced crop competition against fleabane.
- Several years of low winter rainfall resulting in poor winter crop stands and then followed by favourable spring rains for weed germination.
- Poor vegetation growth in non-crop areas due to the recent drought resulting in little competition against fleabane.
- Weed control not specifically targeting fleabane.

*Conyza bonariensis* affects crop production both directly, through plant competition, and indirectly by reducing stored water supplies in fallow via well-developed tap roots, impacting on subsequent crop emergence and growth (Wu et al. 2010). It is a prolific seed producer, and the light seeds are dispersed easily across long distances via both wind and water (Wu 2007). *Conyza bonariensis* acts as a reservoir for insect pests and viruses, including tomato spotted wilt virus in Queensland (Helms et al. 1961). It has also been reported to cause contact dermatitis, as the sap can cause skin irritation (Burry and Kloot 1982).

Populations of *C. bonariensis* have evolved resistance to multiple herbicides, including glyphosate, paraquat, atrazine, simazine, chlorsulfuron and diquat (Bajwa et al. 2016; Heap 2020; Walker et al. 2011). Herbicide-resistant populations require alternative control strategies, such as rotation of different chemical herbicides and integration of cultural control methods (e.g. crop rotation, maintaining good soil fertility) (Bajwa et al. 2016).

## 1.6 Other control methods available

The management of *C. bonariensis* currently involves a combination of chemical and cultural strategies (Wu 2007). Chemical control techniques are most effective when applied to young plants (one to two-month-old rosettes) (Walker et al. 2012). *Conyza bonariensis* can re-sprout when foliage is damaged or removed, which means the weed can be present as several growth stages in field, making the timing of herbicide application difficult (Wu et al. 2010). Herbicide application on mature plants is ineffective due to plant physical characteristics such as high trichome (hair) density, high cuticle thickness and low stomatal density, which limits herbicide uptake (Widderick et al. 2011). For these reasons, a single herbicide application generally results in inconsistent control (Wu 2007; Wu et al. 2008). Therefore, application techniques such as double-knock and residual herbicides are recommended (Widderick et al. 2011; Wu et al. 2006).

Double-knock herbicide application requires that two post-emergent herbicides with differing modes of action are applied to a weed population 5-10 days between one another (Werth et al. 2010; Widderick et al. 2011). Residual herbicides can be applied during fallow to control germinating *C. bonariensis* seed (Widderick et al. 2011). While the use of residual herbicides is effective at minimising the emergence of *C. bonariensis*, these herbicides remain in the soil for a long period, limiting growth of successive crops or pasture species. Planting periods, crop rotation and retention of desirable trees need to be taken into consideration when considering using residual herbicides (Widderick et al. 2011). Continued herbicide use can lead to resistance development in weed populations, as has already been experienced in the northern grain growing region (Walker et al. 2011).

Alternative non-chemical control options recommended for *C. bonariensis* in Australia include decreasing row spacing of crops (Wu and Walker 2004), and strategic use of tillage to invert the soil and bury seed below 2 cm depth (Werth et al. 2008). Unfortunately, these techniques are not always viable options for use on-farm, as they may run contrary to current agricultural practices (e.g. minimum tillage farming adopted to minimise topsoil loss).

## 1.7 Commonwealth, State and Territory legislative controls of the target species

*Conyza bonariensis* is not classified as a noxious weed in Australia (Wu 2007), and to the best of our knowledge no specific state or federal regulatory legislation is applied to the species. It has been listed as a host of *Xylella fastidiosa*, and as such imports of seeds and nursery stock of *C. bonariensis* into Australia are currently restricted (BICON 2020).

## 1.8 Endorsed as a target species for biological control

*Conyza bonariensis* was nominated as a target for biological control by the CSIRO and the nomination was submitted to the Invasive Plants and Animals Committee (now the Environment and Invasives Committee[4]) via the representative from the Queensland Department of Agriculture and Fisheries. It was endorsed as a target for biological control by the Committee in November 2017.[5]

[4] A cross-jurisdictional sectoral sub-committee of the National Biosecurity Committee.

[5] For confirmation, contact Environment and Invasives Committee Secretariat for confirmation eic@agriculture.gov.au.

# 2 Information on proposed biological control agent

## 2.1 Agent name and phylogeny

| | |
|---|---|
| Order: | Pucciniales |
| Family: | Pucciniaceae |
| Genus: | *Puccinia* |
| Species: | *cnici-oleracei* Pers. ex Desm. |
| Pathotype: | ex. *Conyza* |
| Common name: | Flaxleaf fleabane rust |
| Synonyms of *P. cnici-oleracei* [6] | *Puccinia acanthospermi*, *Puccinia asteris*, *Puccinia argentina*, *Puccinia conyzella* [7], *Puccinia diaziana*, *Puccinia doloris*, *Puccinia eleutherantherae, Puccinia emiliae*, *Puccinia melampodii*, *Puccinia ordinata*, *Puccinia picrosiae*, *Puccinia semota*, *Puccinia spilanthis*, *Puccinia synedrellae*, *Puccinia tetranthi, Puccinia wedeliae*, *Puccinia xanthii*, *Puccinia zinnia* |

*Puccinia cnici-oleracei* was first described on *Cnicus oleraceus* (now known as *Cirsium oleraceum*) in France in 1823 (Hennen et al. 2005). Some authors have used the name *P. cnici-oleracei* in a narrow sense for microcyclic rust fungi on *Cirsium* spp., while others have adopted the name broadly for rust species with similar morphology on a range of Asteraceae genera (Berndt 2018). Savile (1970) did not support the broad circumscription of *P. cnici-oleracei* primarily based on host-range differences, and subsequently several new names for similar rust species on Asteraceae hosts were created. *Puccinia doloris* is one of these which has only been recorded on *Conyza*/*Erigeron* species in South America and Central America (Pardo-Cardona 2003).

Hennen et al. (2005) reiterated that *P. cnici-oleracei* is a cosmopolitan complex comprising populations with the same basic morphology that are specialised on different host plants. They considered the different names applied to these different populations as synonyms of *P. cnici-oleracei*, which they wrongly assumed at the time to be the oldest name that applies to this collective morphological species worldwide. Providing this complex is supported with a comprehensive taxonomic investigation, the older published name *Puccinia xanthii* should be used instead of *P. cnici-oleracei* (Hennen pers. comm. 24 July 2007 cited in Seier et al. 2009).

The isolate of the rust fungus that is the subject of this release application, which was purified from an accession collected on a *Conyza* sp. in Colombia, is referred to as *P. cnici-oleracei* (ex. *Conyza*). A voucher herbarium specimen of the isolate will be deposited in the Plant Pathology &

[6] According to Hennen et al. 2005 unless indicated otherwise. Based on types mostly from the Americas.

[7] Described as a new species in 2003 (Pardo-Cardona 2003), but now considered by a Colombian taxonomist as *P. cnici-oleracei*.

Mycology Herbarium of the NSW Department of Primary Industries, Orange, as soon as permission is granted to release the fungus in Australia.

A cross-inoculation experiment with accessions of *P. cnici-oleracei* recovered from a *Conyza* sp. and *Emilia sonchifolia* [8], which were growing in proximity at the same site in Colombia, was performed by our colleagues at the Universidad Nacional de Colombia Sede Medellín to obtain an initial indication of the specificity of the fungus. Both *Conyza* (=*Erigeron*) and *Emilia* species are recorded as hosts of *P. cnici-oleracei* (Farr and Rossman 2020). Healthy and rust-infected plants of the *Conyza* sp. and *E. sonchifolia*, were dug out and transplanted into soil contained in pots. Back at the laboratory, healthy plants of *E. sonchifolia* and the *Conyza* sp. were placed in humid chambers with rust-infected plants of either species at 19°C for 48 h. The inoculated plants were then transferred in a shade house under ambient outdoor conditions, watered daily and examined regularly for symptoms. Plants of the *Conyza* sp., but not of *E. sonchifolia*, developed disease symptoms when exposed to rust infected *Conyza* sp. plants, and vice versa. This demonstrated that the rust accessions were capable of only infecting the host species they originated from.

A phylogenetic analysis comprising a sequence of the ITS1, 5.8s and ITS2 regions of the nuclear rDNA of the purified isolate of *P. cnici-oleracei* (ex. *Conyza*) used in host-specificity testing was performed. It showed that the sequence of *P. cnici-oleracei* (ex. *Conyza*) (Genbank accession no. MT672749) groups with that of another accession of *P. cnici-oleracei* from *Aster* sp. as well as other microcyclic rust fungi with the same broad morphology that occurs on Asteraceae plants: *Puccinia xanthii*, *Puccinia melampodii* and *Puccinia emiliae* (Fig. 4).

[8] The microcyclic rust on *E. sonchifolia* is also reported as *Puccinia emiliae* by some authors (Farr and Rossman 2020).

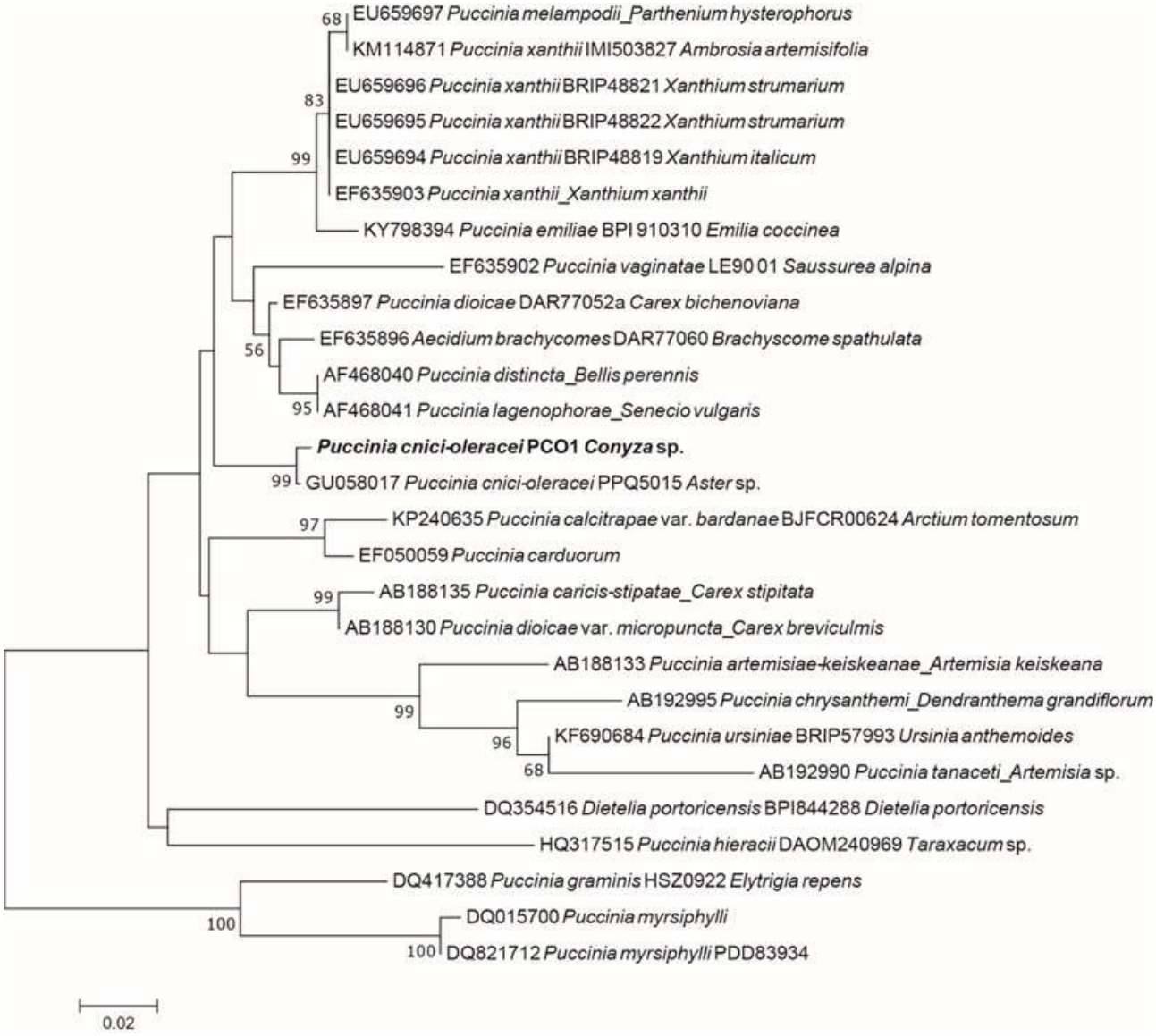


**Figure 4 Phylogenetic relationships of *Puccinia cnici-oleracei* (ex. *Conyza*) and other representative Pucciniales fungi known from Asteraceae using DNA sequence data from the ITS rDNA operon (ITS-1, 5.8S, ITS-2) inferred with the Neighbour-Joining method (Saitou and Nei, 1987) as implemented in MEGA 7 (Kumar et al. 2016). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to nodes. The tree is drawn to scale and rooted with *Puccinia graminis* and *P. myrsiphylli*. *Puccinia cnici-oleracei* (ex. *Conyza*) (PCO1) isolated from *Conyza* sp. in Colombia is highlighted in bold for reference.**

## 2.2 Brief description and biology of the agent

*Puccinia cnici-oleracei* (ex. *Conyza*) is a microcyclic and autoecious rust (i.e. no alternate hosts), with only telia (spermogonia, aecia, and uredinia absent). The telia are dark brown, 0.2-0.3 mm diam., mostly hypophyllous on yellowed spots, in small compact circular groups, sometimes merging into up to 1 cm large cushions. They are present on both young and old leaves, stems and sepals of flower heads (Fig. 5, 6). They are mostly produced on the under surface of leaves, though sometimes developed on the upper side of leaves as the disease progresses (Fig. 6 a, d). Teliospores are two-celled with a thickened wall at the apex, (32-)37-50(-55) x (13-)15-20(-23) µm, narrowly obovoid to obovoid or oblong, constricted at septum, yellow-brown to reddish-brown, smooth, not laminate, pore apical in upper cell, at septum in lower cell (Hennen et al. 2005) (Fig. 5 e, f). Their pedicel is 15-55 µm long and light to dark yellow brown. Teliospores are strongly attached to the telium by their pedicels and are not individually wind-borne. They are capable of germination immediately upon formation, producing an external basidium and four basidiospores. Basidiospores germinate readily on plant tissue, providing some moisture is present, and directly penetrate epidermal cells of susceptible hosts. First visible signs of infection are observed 8 to 9 days after inoculation. Within 15 days of inoculation, telia begin to develop and by 28 days they are well developed (Fig. 6).

## 2.3 Native range of the agent

South and Central America are believed to be the native range of *Puccinia cnici-oleracei* (ex. *Conyza*). The fungus, previous referred to as *Puccinia doloris* (see section 2.4) (Pardo-Cardona 2003; Hennen et al. 2005), has only ever been recorded from *C. bonariensis*, *Erigeron amplexicaule*, *E. bonariensis*, *Erigeron deamii*, *Erigeron hirtellus*, *Erigeron* sp. in Argentina, Chile, Guatemala, Colombia, and Costa Rica (Farr and Rossman 2020).

## 2.4 Species related to the agent and summary of their host range(s)

As explained above (see section 2.1), *P. cnici-oleracei* sensu lato has been recorded on 180 species in the family Asteraceae globally (Farr and Rossman 2020). Some populations of *P. cnici-oleracei* sensu lato have been given different names based on their host specialisation, although they are now all considered as synonyms of *P. cnici-oleracei* by Hennen et al. (2005) (**Error! Reference source not found.**1). Until a comprehensive taxonomic review of the microcyclic rust fungi with the same basic morphology occurring on Asteraceae hosts is undertaken and published, we will be referring to the microcyclic rust fungus specialised on *Conyza* sp. as *P. cnici-oleracei* (ex. *Conyza*).

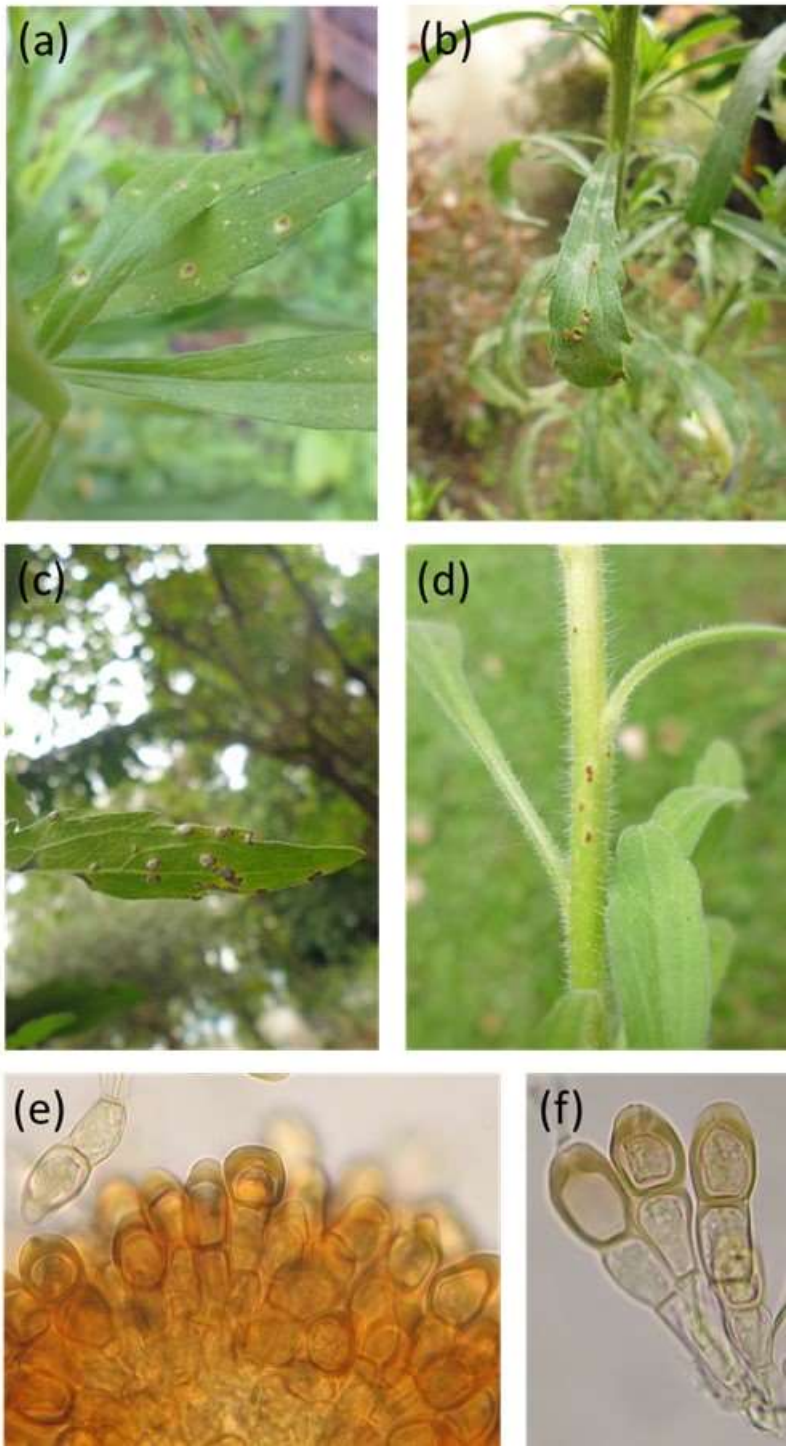


**Figure 5 (a)-(d) Disease symptoms caused by *Puccinia cnici-oleracei* (ex. *Conyza*) observed on *Conyza* sp. in the field in Colombia. Close-up of a telium (e) and teliospores (f). (Photos: Universidad Nacional de Colombia Sede Medellín)**

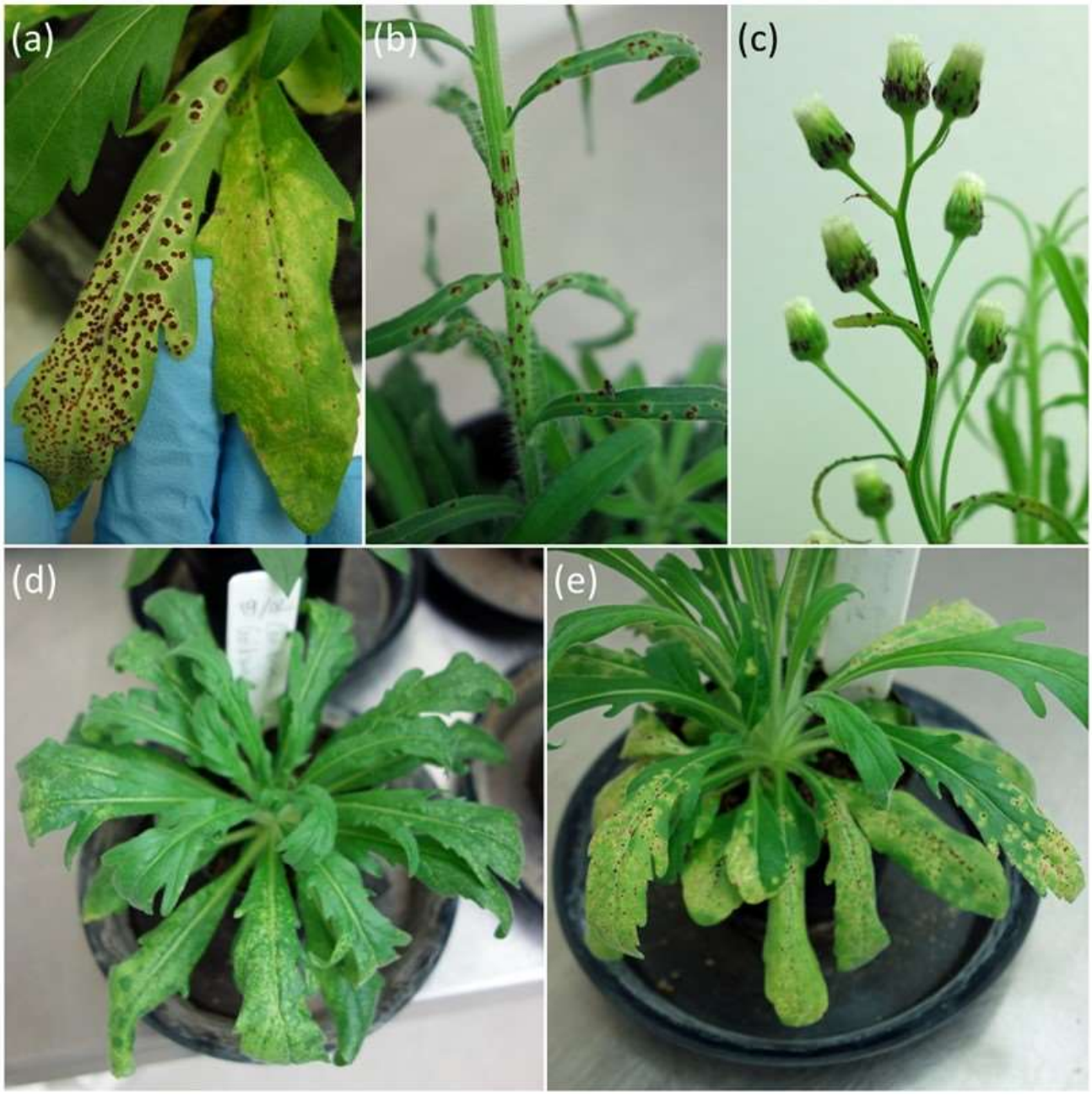


**Figure 6 Disease symptoms caused by *Puccinia cnici-oleracei* (ex. *Conyza*) on *Conyza bonariensis* in the biosecurity containment facility in Australia. Telia on (a) abaxial (left) and adaxial (right) surfaces of leaves, (b) stems and (c) sepals of flowers at 28 days after inoculation. Symptoms on whole plants at (d) 14 and (e) 28 days after inoculation. (Photos: CSIRO)**

**Table 1 Synonyms of *Puccinia cnici-oleracei* sensu lato based on type specimens, mostly from the Americas, that were examined by Hennen et al. (2005).**

| SPECIES | YEAR DESCRIBED | HOST SPECIES OF TYPE SPECIMEN | COUNTRY |
|---|---|---|---|
| *Puccinia xanthii* Schweinitz | 1822 | *Xanthium* sp. | United States |
| *Puccinia asteris* Duby | 1830 | TYPE information not found | – |
| *Puccinia argentina* Spegazzini | 1880 | *Picrosia longifolia* (or *Hieracium* sp.) | Argentina |
| *Puccinia picrosiae* P. Sydow & H. Sydow | 1904 | *Picrosia longifolia* | Brazil |
| *Puccinia doloris* Spegazzini | 1881 | *Erigeron bonariensis* | Argentina |
| *Puccinia spilanthis* P. Hennings | 1892 | *Spilanthes salzmanni* | Brazil |
| *Puccinia melampodii* Dietel & Holway | 1897 | *Melampodium divaricatum* | Mexico |
| *Puccinia synedrellae* P. Hennings | 1898 | *Synedrella nodiflora* | Jamaica |
| *Puccinia emiliae* P. Hennings | 1898 | *Emilia sagittata* | Jamaica |
| *Puccinia acanthospermi* P. Hennings | 1902 | *Acanthospermum xanthioides* | Brazil |
| *Puccinia zinniae* P. Sydow & H. Sydow | 1903 | *Zinnia tennuiflora* | Mexico |
| *Puccinia acanthospermi* H. Sydow & P. Sydow | 1903 | *Acanthospermum xanthoides* | Venezuela |
| *Puccinia diaziana* Arthur | 1905 | *Verbesina encelioides* (*Ximensia encelioides*) | Mexico |
| *Puccinia eleutherantherae* Dietel | 1909 | *Eleutheranthera ruderalis* | Brazil |
| *Puccinia wedeliae* Mayor | 1913 | *Wedelia trichostephia* | Colombia |
| *Puccinia ordinata* H. S. Jackson & Holway | 1918 | *Calea insignis* | Guatemala |
| *Puccinia semota* H. S. Jackson & Holway | 1918 | *Hymenostephium cordatum* (reported as *Gymnolomia subflexuosa*) | Guatemala |
| *Puccinia tetranthi* H. Sydow | 1919 | *Tetranthus literalis* | Haiti |

## 2.5 Proposed source of the agent

All host-specificity experiments performed used a purified (single-telium) isolate of an accession of *P. cnici-oleracei* (ex. *Conyza*) collected in Medellín, Colombia (06°12.918' N, 75°33.668' W). The *Conyza* sp. plant (sample D) on which the rust fungus was collected was molecularly identified as *Conyza sumatrensis* using the DNA chloroplast barcodes *rps16* and *trnQ-UUG* developed by Wang et al. (2018) (Fig. 7).

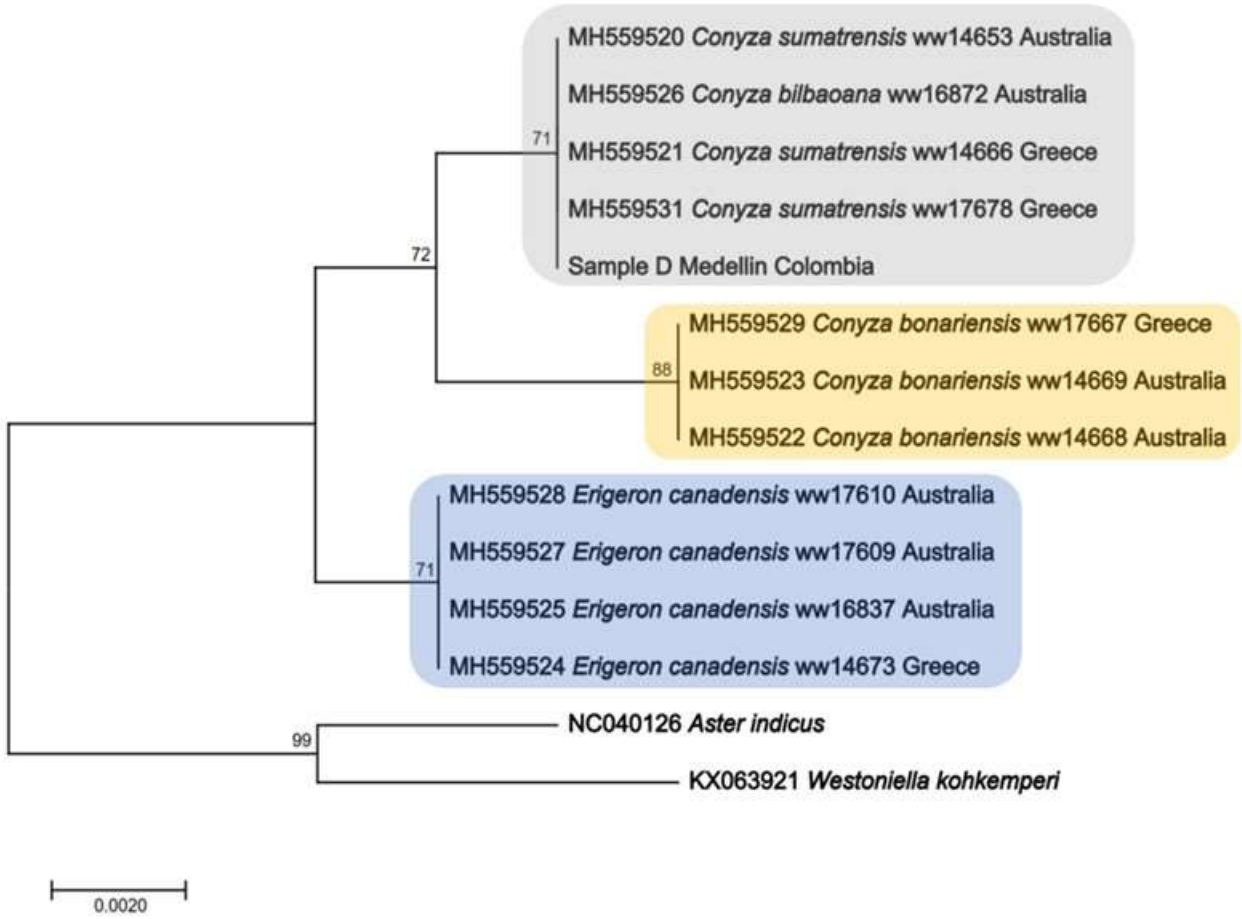


**Figure 7 Phylogram of the sequence from the *Conyza* sp. plant (sample D) on which *Puccinia cnici-oleracei* (ex. *Conyza*) was collected in Medellín, Colombia and GenBank sequences of different *Conyza* species generated using the DNA chloroplast barcodes *rps16* and *trnQ-UUG* developed by Wang et al. (2018). The phylogram was inferred using the Maximum Likelihood method based on the Tamura 3-parameter model (Tamura 1992). The phylogram with the highest log likelihood (-853.54) is shown and the percentage of trees in which the associated species clustered together, following 1000 Bootstrap replicates, is shown at branch nodes. Analyses were conducted in MEGAX (Kumar et al. 2018). Note that *Erigeron canadensis* is a synonym of *Conyza canadensis*, the name that is widely used in Australia.**

## 2.6 Agent's potential for control of the target

*Puccinia cnici-oleracei* (ex. *Conyza*) infects both young and old leaves, stems and sepals of flowerheads of *C. bonariensis*. It obtains nutrients and water from its host plant by establishing an intimate contact with living cells. Through this continuous absorption and diversion of assimilates from the plant, the fungus restricts plant development and reproduction (Fig. 8). The fungus also destroys these tissues by producing fruiting bodies, thus reducing the photosynthetic surface area of the plant.

Other microcyclic rust fungi of weeds, such as *Puccinia xanthii* on Noogoora burr (*Xanthium occidentale*) (Morin et al. 1996) in Australia, and *Puccinia spegazzinii* on mikania (*Mikania micrantha*) introduced in nine other countries (Day et al. 2013) have proved to be very effective biological control agents.

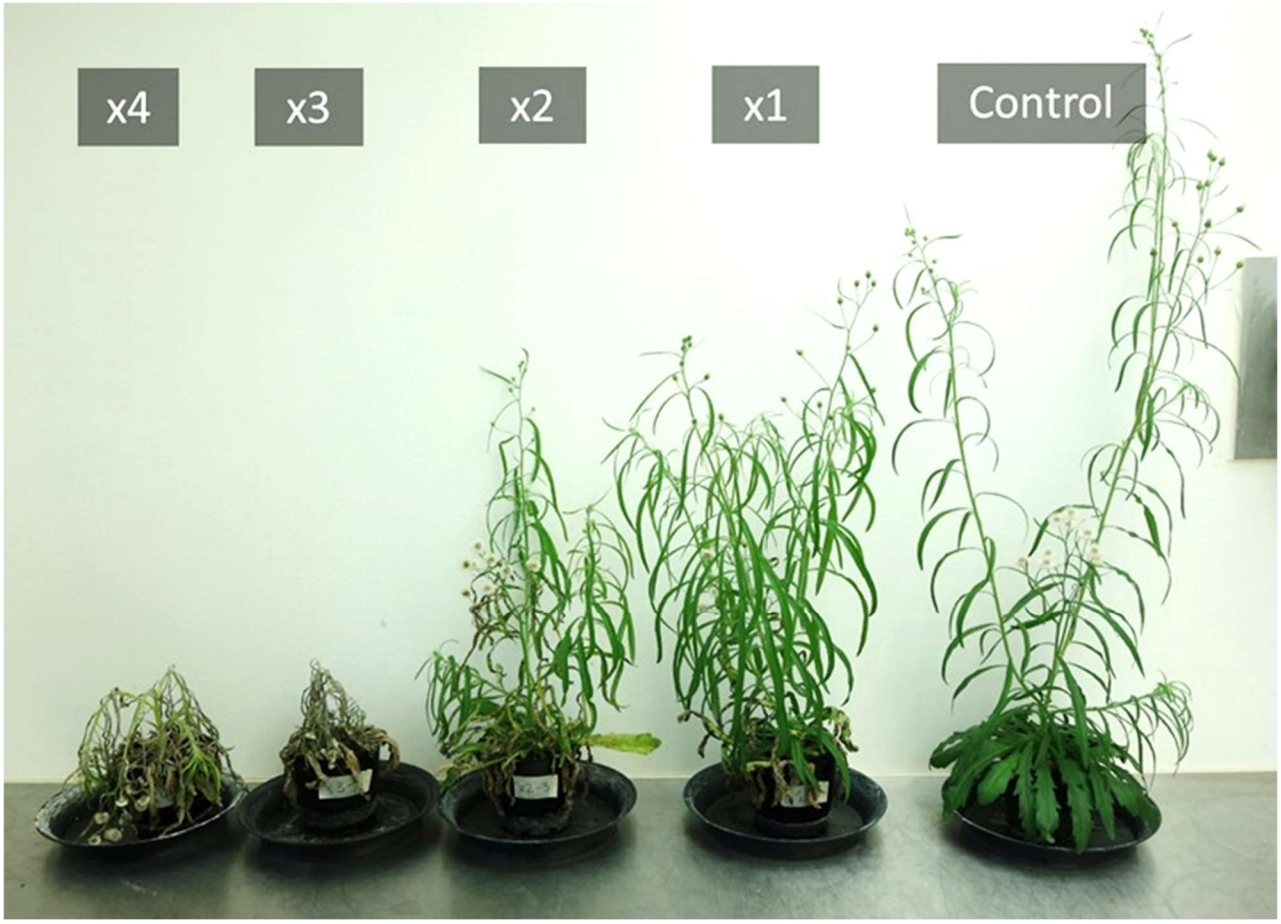


**Figure 8 Example of impact of *Puccinia cnici-oleracei* (ex. *Conyza*) on *Conyza bonariensis* (accession Con_1 (C2)). Young plants were artificially inoculated one, two, three or four times under controlled environment conditions in the biosecurity containment facility over a 6-week period. Inoculations were performed every fortnight. Control plants were not inoculated. Photo taken at 2 weeks after the last inoculation.**

## 2.7 Information on non-target organisms at risk from the agent

*Conyza* is mostly a New World genus of the family Asteraceae, the largest of all plant families (c. 25,000 species worldwide). The Asteraceae in Australia comprises over 1,000 native species and hundreds of introduced species, many of which have become naturalised (Brown 2014). *Conyza* belongs to the subfamily Asteroideae, tribe Astereae, subtribe Conyzinae and is considered to have arisen from the genus *Erigeron* (Nesom 2008). There are no *Conyza* species native to Australia. Following a taxonomic revision, there is only one native species in the genus *Erigeron* recognised in Australia; *Erigeron conyzoides* (Nesom 1994).

There are also several Australian native genera outside the subtribe Conyzinae in the tribe Astereae such as in the *Brachyscome*, *Olearia* and *Vittadinia* groups (Xiaoping and Bremer 1993). It is noteworthy that there are no economic crops grown in Australia that belong to the tribe Astereae, although there are many species that have been introduced for horticultural purposes, some of which are reported as weeds in Australia and elsewhere (Randall 2007). The tribes most closely related to Astereae in the subfamily are Anthemideae, Gnaphalieae, Calenduleae and Senecioneae (Appendix A). Sunflower (*Helianthus annuus*) is an important crop plant that belongs to tribe Heliantheae, which is more distant to Astereae.

## 2.8 Similar host-specificity assessments undertaken with the agent

Our collaborators in Colombia indicated that the accession of *P. cnici-oleracei* (ex. *Conyza*) collected in Medellín, which we imported in the biosecurity containment facility, developed on three *Conyza* accessions from Colombia in glasshouse tests. Abundant telia developed on an accession putatively identified as *C. bonariensis* using Wang et al. (2018) barcodes, while only scarce telia developed on accessions identified as *C. sumatrensis* var. *sumatrensis* and *C. sumatrensis* var. *leiotheca* (identified by an expert taxonomist on Asteraceae from the Missouri Botanical Garden). Beside these preliminary tests, no other risk assessment of a population of *P. cnici-oleracei* from *Conyza* sp., other than that presented in this document, have been undertaken before.

Host-specificity assessments of *Puccinia xanthii* var. *parthenii-hysterophorae* (previously known as *Puccinia melampodii*; Seier et al. 2009), which belongs to the collective morphological species *P. cnici-oleracei* sensu lato according to Hennen et al. (2005), were performed prior to its release in Australia being approved for the biological control of *Parthenium hysterophorus* (Seier 1999). These assessments showed that the fungus has a restricted host-range, developing severe disease symptoms only on *P. hysterophorus* and limited infections on a few other species, including sunflower. Following its release, a field experiment, comprising all plants on which some symptoms developed under laboratory conditions, was conducted to confirm the host range under natural conditions (Tomley 2000). Development of small telia was only observed on *Calendula officinalis*. There have been however, no records of non-target damage in the wild since the release of this rust fungus in Australia (Barton 2012).

Host-specificity assessments have also been undertaken post-introduction on another population of *P. xanthii* specialised on *Xanthium* spp. weeds (Alcorn 1976; Morin et al. 1993). This fungus was first recorded in Australia in 1974 following an unauthorised introduction (Alcorn 1975). Some infection occurred on *C. officinalis* and a few cultivars of sunflower in those laboratory tests. Telia were also observed on some sunflower cultivars in the field soon after the first record of the fungus (Alcorn and Kochman 1976). However, as predicted by Kochman (1980), the fungus has not had any impacts on the sunflower industry in Australia.

## 2.9 Possible interactions, including conflicts with existing biological control programs

Biological control agents released for *C. bonariensis* in Australia will primarily affect populations of the weed in non-disturbed land adjacent to cultivated fields, thus reducing propagule pressure on cropping systems at a landscape scale.

No biological control agents have been released for *C. bonariensis* in Australia, and therefore no conflicts with other biological control programs for this weed is expected at this stage. However, native range surveys for candidate biological control agents in Colombia and Brazil have identified promising candidate insect agents which are being investigated further.

## 2.10 Where, when and how initial releases will be made

Upon obtaining approval to release *P. cnici-oleracei* (ex. *Conyza*) in Australia, large numbers of *C. bonariensis* plants, maintained in the CSIRO glasshouses at Black Mountain, Canberra, will be inoculated with plant material bearing telia removed from the biosecurity containment facility (in the presence of relevant officers from the Department of Agriculture, Water and the Environment). Infected *C. bonariensis* plants from these glasshouses will then be used to establish infections in the field at selected sites across the weed's range, with a focus on the Northern and Western grains growing regions (as defined by the GRDC). Sites will be selected in consultation with grains weeds experts, the GRDC and grower groups from these regions. Disease development and spread will be closely monitored during the first few growing seasons, provided funding is available. Redistribution of *P. cnici-oleracei* (ex. *Conyza*) from infected to noninfected sites may be necessary given that the fungus' basidiospores are fragile and not known to travel long distance on wind currents.

## 2.11 Established populations of the agent: where, mode of introduction, spread and any off-target impacts recorded

*Puccinia cnici-oleracei* (ex. *Conyza*) has not been reported outside its native range in South and Central Americas.

## 2.12 Host-specificity testing in the biosecurity containment facility

### 2.12.1 Test list

The list of non-target plant species (51) used to test the specificity of *Puccinia cnici-oleracei* (ex. *Conyza*) was compiled according to the phylogenetic centrifugal approach, which places greater representation on the more closely related species to the target weed (Wapshere 1974; Briese 2003) (Table 2). Within this phylogenetic/evolutionary framework, selection of representative test species places an emphasis on endemic species, species of economic importance and those that are likely to overlap biogeographically with the target weed, where possible. No unrelated crop species were included in the test list since these species do not make any contribution to the delineation of the host range of specialised biological control agents (Briese 2003; Sheppard et al. 2005).

Recent published molecular phylogenies of Asteraceae (Brouillet et al. 2009; Li et al. 2012; Fu et al. 2016; Stevens 2001) were used to devise the test list so that species most closely related to *C. bonariensis* that are present in Australia were given priority. The test list comprised many species within the Astereae, the tribe to which *C. bonariensis* belongs to (**Error! Reference source not found.**2). The list also included representative species in another 15 tribes related to Astereae within the subfamily Asteroideae (Appendix A), that are present in Australia.

**Table 2 List of plant species used to test the specificity of *Puccinia cnici-oleracei* (ex. *Conyza*) in the biosecurity containment facility in Australia. All species are within the subfamily Asteroideae in the family Asteraceae.**

| TRIBE | SUBTRIBE | RELATIONSHIP TO TARGET WEED | | PLANT SPECIES | STATUS IN AUSTRALIA [1] |
|---|---|---|---|---|---|
| Astereae | Conyzinae | Target weed | 1 | *Conyza bonariensis* | Weed |
| | | Same genus | 2 | *Conyza canadensis* | Weed |
| | | | 3 | *Conyza sumatrensis* | Weed |
| | | Same subtribe | 4 | *Erigeron karvinskianus* | Ornamental |
| | | | 5 | *Erigeron glaucus* | Ornamental |
| | | | 6 | *Erigeron speciosus* | Ornamental |
| | | | 7 | *Erigeron speciosus* "Quakeress" | Ornamental |
| | Chrysopsidinae | Same tribe | 8 | *Heterotheca grandiflora* | Weed |
| | Solidagininae | | 9 | *Solidago canadensis* | Naturalised |
| | Symphyotrichinae | | 10 | *Symphyotrichum novi-belgii* | Weed |
| | | | 11 | *Aster cordifolius* | Ornamental |
| | Machaerantherinae | | 12 | *Grindelia camporum* | Naturalised |
| | | | 13 | *Grindelia robusta* | Ornamental |
| | Grangeinae | | 14 | *Eschenbachia leucantha* | Naturalised |
| | Hinterhuberinae | | 15 | *Olearia phlogopappa* | Native |
| | | | 16 | *Olearia minor* | Native |
| | | | 17 | *Olearia floribunda* | Native |
| | Podocominae | | 18 | *Vittadinia muelleri* | Native |
| | Lagenophorinae | | 19 | *Pappochroma bellidioides* | Native |
| | Baccharidinae | | 20 | *Baccharis halimifolia* | Weed |
| | Brachyscominae | | 21 | *Brachyscome multifida* | Native |
| | Bellidinae | | 22 | *Bellis perennis* | Naturalised |
| | Homochrominae | | 23 | *Felicia amelloides* | Ornamental and Weed |
| Anthemideae | Artemisiinae | Same subfamily | 24 | *Chrysanthemum maximum* | Ornamental |
| | Glebionidinae | | 25 | *Glebionis coronaria* | Weed |
| | Anthemidinae | | 26 | *Tanacetum vulgare* | Weed |
| Gnaphalieae | | | 27 | *Cassinia longifolia* | Native |
| | | | 28 | *Xerochrysum bracteatum* | Native |
| | | | 29 | *Xerochrysum viscosum* | Native |
| | | | 30 | *Ozothamnus diosmifolius* | Native |
| | | | 31 | *Ozothamnus secundiflorus* | Native |
| Calenduleae | | | 32 | *Calendula officinalis* | Ornamental & Weed |
| | | | 33 | *Dimorphotheca pluvialis* | Ornamental & Weed |

| TRIBE | SUBTRIBE | RELATIONSHIP TO TARGET WEED | | PLANT SPECIES | STATUS IN AUSTRALIA [1] |
|---|---|---|---|---|---|
| | | | 34 | *Dimorphotheca sinuata* | Ornamental & Weed |
| Senecioneae | | | 35 | *Senecio pinnatifolius* ssp. *pinnatifolius* | Native |
| | | | 36 | *Senecio pinnatifolius* var. *alpinus* | Native |
| | | | 37 | *Abrotanella forsteroides* | Native |
| | | | 38 | *Bedfordia arborescens* | Native |
| Inuleae | | | 39 | *Streptoglossa bubakii* | Native |
| Athroismeae | | | 40 | *Centipeda minima* | Native |
| Helenieae | | | 41 | *Gaillardia grandiflora* | Naturalised |
| | | | 42 | *Gaillardia pulchella* | Naturalised |
| Coreopsideae | | | 43 | *Bidens pilosa* | Weed |
| Neurolaeneae | | | 44 | *Enydra woollsii* | Native |
| Tageteae | | | 45 | *Tagetes erecta* | Ornamental |
| | | | 46 | *Tagetes patula* | Ornamental |
| Bahieae | | | 47 | *Schkuhria pinnata* | Weed |
| Heliantheae | | | 48 | *Helianthus annuus* | Crop |
| Millerieae | | | 49 | *Guizotia abyssinica* | Naturalised |
| Eupatorieae | | | 50 | *Adenostemma lavenia* | Native |
| | | | 51 | *Ageratina adenophora* | Weed |
| Madieae | | | 52 | *Arnica chammissonis* | Naturalised |

[1] As recorded in Randall (2007) or the Australian Plant Census (https://biodiversity.org.au/nsl/services/APC).

The original proposed test list comprised 41 species. However, additional species were added to this list, including some to replace species that could not be readily sourced. All alternative species included are within the same tribe as the originally listed species.

There are records of eight *Erigeron* species occurring in Australia in the Atlas of Living Australia (ALA)[9], which follows the broad taxonomic circumscription and uses *Erigeron* as the accepted genus name for species we refer to as *Conyza* in our test list (Table 3). Note that one of these listed species, *Erigeron pappochromus* is a misapplied name and its correct name is *Pappochroma bellidioides*, a native species in Australia that we included in our tests as a representative of this genus. The New South Wales Flora online [10] lists an additional four *Erigeron* species; *E. bellidioides*, *E. nitidus*, *E. paludicola* and *E. setosus* which are considered in ALA as synonyms of the accepted names in the *Pappochroma* genus; *P. bellidioides*, *P. nitidum*, *P. paludicola* and *P. setosum.* The Flora of Victoria [11] also lists the first three of these *Pappochroma* species, which occur in that state.

[9] https://www.ala.org.au/

[10] https://plantnet.rbgsyd.nsw.gov.au/floraonline.htm

[11] https://vicflora.rbg.vic.gov.au/

**Table 3 *Erigeron* species present in Australia according to the Atlas of Living Australia (ALA) and information on their inclusion in our host-specificity testing with *Puccinia cnici-oleracei* (ex. *Conyza*).**

| ALA RECORD | STATUS IN AUSTRALIA [1] | SPECIES INCLUDED IN HOST-SPECIFICITY TESTING | COMMENT |
|---|---|---|---|
| *Erigeron bilbaoanus* (=*Conyza bilbaoana*) | Introduced - Weed | No | We approached two Australian taxonomists with experience with *Conyza* and both comments that they would not be confident to identify this species in the field. Consequently, it could not be sourced for our tests. |
| *Erigeron bonariensis* (=*Conyza bonariensis*) | Introduced - Weed | Yes - Target weed | |
| *Erigeron canadensis* [2] (=*Conyza canadensis*; *Conyza parva*) | Introduced - Weed | Yes | |
| *Erigeron conyzoides* | Native | No | We sought the help of several individuals from a range of organisations to source this rare species but was not successful (Appendix B). |
| *Erigeron karvinskianus* | Ornamental | Yes | |
| *Erigeron primulifolius* (=*Conyza primulifolia*) | Introduced - Weed | No | Distribution restricted compared to that of *C. bonariensis*, C*. sumatrensis* and *C. canadensis.* Could not be sourced. |
| *Erigeron sumatrensis* (=*Conyza sumatrensis*) | Introduced - Weed | Yes | |
| **Misapplied name:** *Erigeron pappochromus* (accepted name *Pappochroma bellidioides*) | Native | Yes | |

[1] As recorded in Randall (2007) or the Australian Plant Census (https://biodiversity.org.au/nsl/services/APC).
[2] Listed as *Erigeron pusillus* in Flora of Victoria (https://vicflora.rbg.vic.gov.au/).

It is noteworthy that we included in our host-specificity testing two additional *Erigeron* species that are not recorded in ALA and other Australian herbaria but that are sold as ornamentals in Australia: *Erigeron glaucus* and *Erigeron speciosus*.

## 2.12.2 Materials and methods

### 2.12.2.1 Plant production

*Conyza bonariensis* plants used in each experiment were grown from seed collected at Abercrombie, NSW (33°55'41.98"S, 149°21'5.96"E). Seeds were sown into 1:1 perlite and vermiculite mixture, and seedlings pricked out and placed into potting mix (5:1:1:3 straw-based compost, peat moss, river sand, perlite, with 1.4 kg slow-release fertilizer per $m^3$ [Aboska®, N:P:K 15.16:6.93:5.19]) in 5 cm diam. plastic pots. All *C. bonariensis* plants were grown in controlled-temperature (CT) rooms at 23°C with a 14-h photoperiod provided by plant LED growth lights.

The various non-target plant species used in the experiments were propagated either from seeds, cuttings or whole plants obtained from commercial outlets or from the field and grown in the glasshouse or CT rooms. Actively growing plants were taken into the biosecurity containment facility prior to each experiment.

All plants were fertilised fortnightly with Aquasol (Yates, Clayton, Vic., Australia; NPK 23:3.95:14), and treated as necessary with pesticides (never fungicides; most commonly Confidor, Yates, Caltyon, Vic., Australia [15mg/L Imidacloprid] as a soil drench, or Vertimec, Syngenta, Macquarie Park, NSW, Australia [18 g/L Abamectin] as a spray) to reduce pest pressure. All plants treated with pesticides were withheld from experiments for a minimum of two weeks following application to reduce the likelihood of the pesticide interacting with fungal infection.

### 2.12.2.2 Production of inoculum

Every two to four weeks, five *C. bonariensis* plants (at least four weeks old, with at least six expanded leaves) were inoculated with *P. cnici-oleracei* (ex. *Conyza*) with a method previously used with other microcyclic rust fungi (e.g. Morin et al. 1993; Seier 1999), to maintain a continuous supply of inoculum for experiments. Leaf pieces (~1 $cm^2$) with at least 50 % coverage of mature telia were cut from infected *C. bonariensis* leaves (approx. 4 wks after inoculation) and each deposited onto the slightly melted surface of a 2% water agar block (1–2 $cm^2$) placed in the base of a 9 cm diam. Petri dish (telia uppermost; three blocks per dish, unless indicated otherwise, placed evenly around the dish) (Fig. 9). The dishes containing telia (without lids) were then fixed with sticky tape to the inside bottom of 10 L opaque plastic buckets (one dish per bucket). Each bucket with telia was inverted over the opening of another 10 L bucket containing a single *C. bonariensis* plant that had been misted with distilled water. The buckets were placed in a CT room at 20 °C for 48 h. During this period teliospores germinated and produced basidiospores that were naturally discharged onto the plants' foliage. Plants were then removed from the buckets and placed on the bench of the CT room (12 h photoperiod, fluorescent lights).


**Figure 9 Methods used to inoculate plants with *Puccinia cnici-oleracei* (ex. *Conyza*). (a) Water agar blocks with *Conyza bonariensis* leaf pieces with mature telia placed in a Petri dish without lid (inset) that was fixed to the base of a bucket which was inverted above the non-target plant to be inoculated. (b) Set-up used to inoculate a single leaf of a non-target plant with telia of the fungus.**

#### 2.12.2.3 Experimental design

Each plant species was tested in at least two separate experiments, unless otherwise indicated. Five replicate plants per species/accession were generally used in each experiment (although in some cases three, four or six replicates were used). In most instances, different accessions of the species were included in different experiments to account for any possible variation in time and provenance of the plant material (Appendix C). Actively growing plants (up to 30 cm in height, including pot) were chosen for each experiment. Each experiment consisted of 2–8 species, including the positive control *C. bonariensis* (accession Con_1 (C2)). Randomized complete block designs at the plant level were used in all experiments to reduce the influence of random factors such as lighting within the CT room and the quality and consistency of inoculum. Each block contained a replicate of each species, which were grouped together on the bench of the room.

#### 2.12.2.4 Inoculations

Inoculations with *P. cnici-oleracei* (ex. *Conyza*) were performed as described in section 2.12.2.2, unless indicated otherwise. Telia used to inoculate each plant were visually checked after the inoculation chambers were dismantled 48 hours after the initiation of each experiment. Telia with germinated teliospores are readily identified by their whitish appearance because of the intertwined mat of germ-tubes/basidia produced. An experiment was considered valid if all inoculated *C. bonariensis* plants had developed telia, with at least three of the replicates with a rating of at least 4 at the end of the experiment (Table 4).

**Table 4 Rating system used to assess visible symptoms on plants, including the control *Conyza bonariensis*, inoculated with *Puccinia cnici-oleracei* (ex. *Conyza*) in each host-specificity experiment.**

| RATING | SYMPTOMS |
|---|---|
| 0 | No visible symptoms. |
| 1 | Chlorotic, purplish or necrotic flecks or blotches only. |
| 2 | Very few and small or under-developed telia (< 1 mm diameter), associated with or without chlorosis or necrosis. |
| 3 | Fully developed, normal sized telia (> 1 mm diameter), covering less than 25% of leaf surface area. |
| 4 | Fully developed, normal sized telia (> 1 mm diameter), covering 25 - 50% of leaf surface area. |
| 5 | Fully developed, normal sized telia (> 1 mm diameter), covering 50 - 75% of leaf surface area. |
| 6 | Fully developed, normal sized telia (> 1 mm diameter), covering more than 75% of leaf surface area. |

Additional inoculations targeted at single leaves of test plants were performed during the experiments to provide material for microscopic examination of development of the rust fungus. Leaf pieces (0.5–1 $cm^2$) with three or more mature telia of *P. cnici-oleracei* (ex. *Conyza*) were cut from infected *C. bonariensis* leaves (approx. 4 weeks after inoculation), then cut in half or thirds before each piece was placed on an agar block, as described in section 2.12.2.2. Two blocks with telia were placed in 5 cm diam. petri dishes (telia uppermost). The two dishes with telia (without lids), each attached to a fine bamboo stick with a metal clip, were then inverted above a single leaf or group of leaves (when very small) on one plant of each test species (Fig. 9 b). Narrow strips of sticky tape were used, if necessary, to ensure the dish with telia remained lined up with the leaf for the duration of the inoculation. The plant was then placed in a 10 L opaque plastic bucket, misted with distilled water, covered with another 10 L bucket and placed in a CT room (conditions as above). After 48 h, the inoculation set-up was dismantled, and the plant was removed from the bucket and placed on the bench of the CT room.

### 2.12.2.5 Microscopic examinations

For each plant species (one or more accessions), one of the inoculated leaves or group of small leaves was excised 5–6 days after inoculation and cut into small pieces (0.5–1 $cm^2$). Where chlorotic flecks or necrotic blotches developed on plants during the experiments, additional samples were taken to examine the interaction between the fungus and plant in relation to the symptoms.

The pieces were cleared and stained in a solution containing aniline blue, ethanol, chloroform, lactic acid, phenol and chloral hydrate for 24–48 h (and in a few instances > 72 h) (Bruzzese and Hasan 1983). They were then rinsed in water, placed in a saturated solution of chloral hydrate for 24 h (and in rare instances 48 h) and transferred back to water for storage. Prior to microscopic examination the pieces were placed in blue-lacto-glycerol stain on a microscope glass slide for 3–5 min. Excess stain was then gently removed with blotting paper and pieces were mounted in water and examined under a light microscope. Up to 50 basidiospores per accession inoculated were examined. Percentage germination was assessed for some of the accessions. In instances where no basidiospores could be located on the samples processed, the species/accession was inoculated again, and samples taken as described above.

#### 2.12.2.6 Assessment of *P. cnici-oleracei* (*ex. Conyza*) development and plant response

The microscopic development of *P. cnici-oleracei* (ex. *Conyza*) and reproduction on test plants were assessed according to 19 categories (Fig. 10). Plants were examined at 14 and 27–29 days after inoculation for the presence of visible symptoms and rated (Table 4). The overall response of each species/accession to the fungus was classified according to one of seven categories (Table 5), by considering microscopic observations (Fig. 10) and the most severe visible symptoms observed across all replicate plants (Table 4). Species/accessions classified as Moderately Susceptible were kept for another 28 days to determine if any of the underdeveloped, non-eruptive and/or minuscule telia developed any further. Wherever present, these minuscule telia were used to inoculate single leaves of *C. bonariensis* following the method described above for single leaf inoculation. Concurrently, telia of a similar age from *C. bonariensis* were used to inoculate other *C. bonariensis* plants to provide a control. Leaves were assessed for presence of telia at 28 days after inoculation.

### 2.12.3 Results

In all experiments, clear signs of germinated teliospores were observed on telia used to inoculate individual plants when the inoculation chambers were dismantled, indicating that basidiospores had been released onto the test plants.

#### 2.12.3.1 Microscopic development and reproduction of *P. cnici-oleracei* (ex. *Conyza*) on tested species

The full range of normal microscopic developmental stages of *P. cnici-oleracei* (*ex. Conyza*) (Fig. 10) were observed on *C. bonariensis*, *Eschenbachia leucantha* and *Vittadinia muelleri* (Table 6, Figs 11, 12, 13). Normal telia however, only developed on *C. bonariensis*. *Eschenbachia leucantha* was the only species in which the fungus proceeded to produce a few pin-sized telia on one and two replicate plants in each of the experiments performed with this species (Fig. 14). Chlorotic and necrotic flecks were also observed on these and other replicate plants in these experiments. These minuscule telia did not develop any further in the weeks following their first appearance. Following inoculation of *C. bonariensis* with these telia, only one leaf exposed to a single telium produced on *E. leucantha* in the second experiment, became infected and developed a few telia.

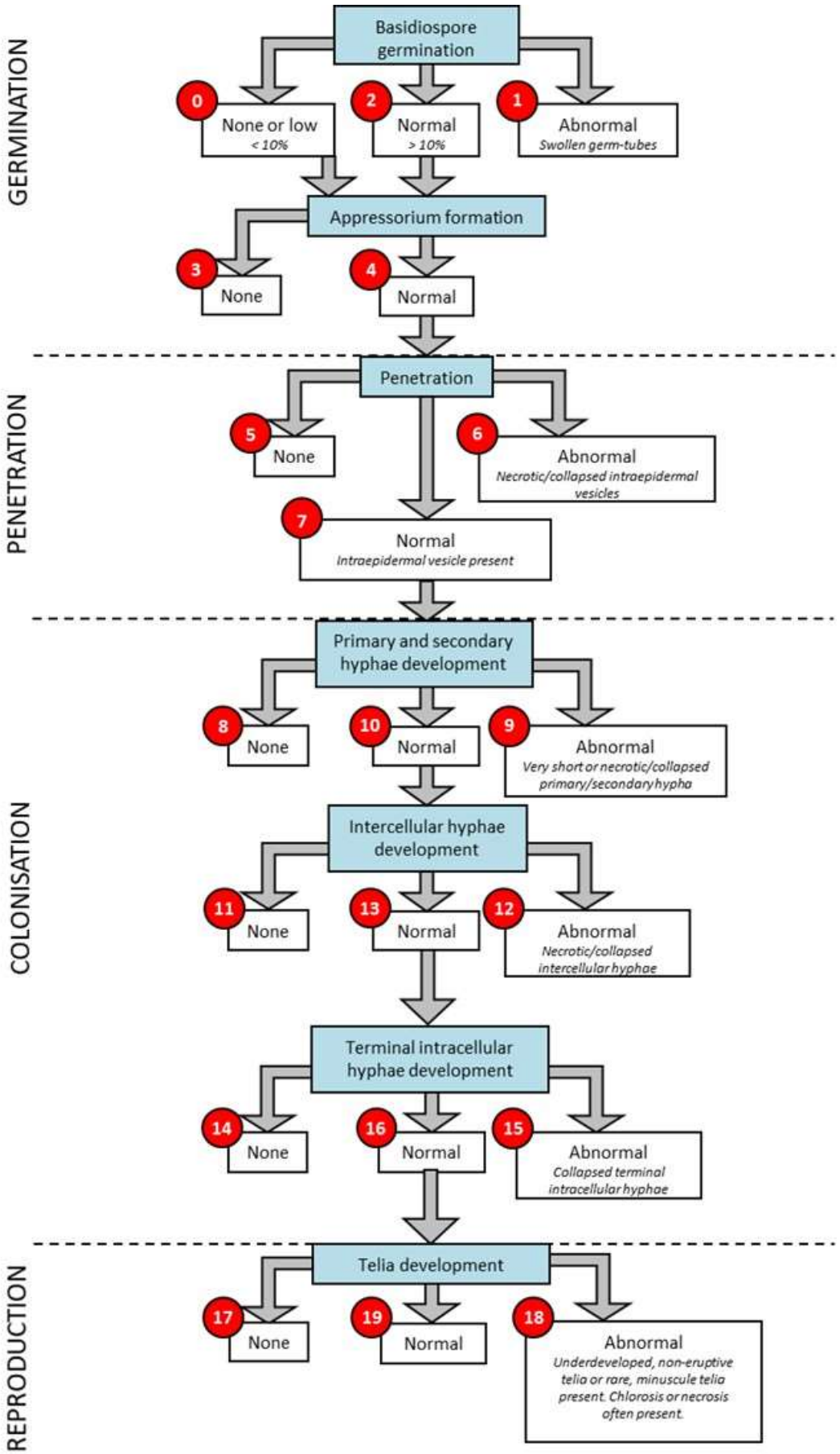


**Figure 10 Schematic representation of the categories used to assess microscopic development and reproduction of *Puccinia cnici-oleracei* (ex. *Conyza*) on the test plant species.**

**Table 5 Categories used to classify the response of plant species to *Puccinia cnici-oleracei* (ex. *Conyza*).**

| CATEGORY | VISIBLE SYMPTOMS | TYPICAL DEVELOPMENTAL STAGE OF THE FUNGUS FOR THE CATEGORY [1] |
|---|---|---|
| **Immune (I)** | None | No sign of penetration |
| **Highly resistant (HR)** | None | Abnormal penetration (intraepidermal vesicle necrotic/collapsed or normal penetration with primary hypha either very short, necrotic/collapsed or absent); plant defence reaction sometimes visible at the cellular level. |
| **Resistant (R)** | Discolouration, chlorosis and/or chlorotic or necrotic flecks sometimes present. | Successful penetration and development of primary hypha and some intercellular hyphae. No terminal intracellular hyphae present. |
| **Moderately resistant (MR)** | Chlorotic or necrotic spots or blotches present. Chlorotic or necrotic flecks may also be present. | Restricted network of intercellular hyphae; terminal intracellular hyphae present, but generally collapsed. Plant host cell plasmolysis often present. |
| **Moderately susceptible (MS)** | Underdeveloped, non-eruptive telia or rare, minuscule telia present. Chlorosis or necrosis, including flecks, often present. | Extensive network of infection hyphae; terminal intracellular hyphae abundant but often collapsed. Development of telia initiated but aborted, incomplete or restricted. |
| **Susceptible (S)** | Normal-sized telia present but restricted in numbers. | Extensive network of intercellular hyphae; abundant, well-developed terminal intracellular hyphae. |
| **Highly susceptible (HS)** | Large number of normal-sized telia present. | Extensive network of intercellular hyphae; abundant, well-developed terminal intracellular hyphae. |

[1] In some instances microscopic development of the fungus was either less or more advanced than the typical development stage associated with the category used to classify the plant response.

Basidiospores of *P. cnici-oleracei* germinated on most non-target species, producing germ-tubes of different length, each with or without an appressorium, but the fungus did not penetrate plant epidermal cells (Table 6). In a few instances, germinated basidiospores were associated with putative defence reactions by the plant (Fig. 15). Abnormal and often collapsed intraepidermal vesicles were observed in *Conyza sumatrensis*, *Xerochrysum bracteatum*, *Bellis perennis*, *Gaillardia pulchella*, *Enydra woollsii* and *Adenostemma lavenia* (Table 6, Fig. 16). The fungus only produced primary hyphae that were short, necrotic and/or collapsed in *Baccharis halimifolia, Calendula officinalis*, *Dimorphotheca sinuata, Senecio pinnatifolius var. alpinus* and *Ozothamnus diosmifolius* (Table 6, Fig. 16). Normal primary hyphae were observed in one accession of *B. halimifolia*, but the intercellular hyphae that developed were abnormal and restricted.

**Table 6 Microscopic development and reproduction of *Puccinia cnici-oleracei* (ex. *Conyza*) on each of the test plant species/accessions inoculated with the fungus, based on categories described in Figure 10. A plus sign (+) indicates that this category was observed. The overall response of each species/accession was determined by combining these results with those presented in Table 7 and classified using categories presented in Table 5.**

| SPECIES [1] | ACCESSION | EXP. (NO. REPS) | MICROSCOPIC EXAMINATION | | | | | | | | | | | | | | | | | | | VISIBLE EXAMINATION | | | OVERALL SPECIES RESPONSE [2] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | GERMINATION | | | | | PENETRATION | | | COLONISATION | | | | | | | | | | | REPRODUCTION | | | |
| | | | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | |
| *Conyza bonariensis* | Con_1 (C2) | All [3] | - | - | + | - | + | - | - | + | - | - | + | - | - | + | - | - | + | - | - | + | HS |
| | CB-Goldsworth | E (5) | Microscopic development not assessed | | | | | | | | | | | | | | | | | - | - | + | HS |
| | Q.Con.bon_3 | E (5) | Microscopic development not assessed | | | | | | | | | | | | | | | | | - | - | + | HS |
| | Con.bon_1 | N (5) | Microscopic development not assessed | | | | | | | | | | | | | | | | | - | - | + | HS |
| | Con.bon_6 | N (5) | Microscopic development not assessed | | | | | | | | | | | | | | | | | - | - | + | HS |
| | Con.bon_7 | N (5) | Microscopic development not assessed | | | | | | | | | | | | | | | | | - | - | + | HS |
| | Con.bon_8 | N (5) | Microscopic development not assessed | | | | | | | | | | | | | | | | | - | - | + | HS |
| | Con.bon_9 | N (5) | Microscopic development not assessed | | | | | | | | | | | | | | | | | - | - | + | HS |
| | Con.bon_10 | N (5) | Microscopic development not assessed | | | | | | | | | | | | | | | | | - | - | + | HS |
| *Conyza canadensis* | Con.can_1 | A (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| | Con.can_1 | R (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| *Conyza sumatrensis* | Con.sum (C1, C3) | G (5) | - | - | + | - | + | - | + | - | - | - | - | - | - | - | - | - | - | + | - | - | MR |
| | Con.sum (C1, C3) | V (5) | - | - | + | - | + | - | + | - | - | - | - | - | - | - | - | - | - | + | - | - | MR |
| | Con.sum_3 | H (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | MR |
| | Con.sum_4 | F (5) | - | - | + | - | + | - | + | - | - | - | - | - | - | - | - | - | - | + | - | - | MR |
| | Con.sum_6 | I (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | R |
| | Con.sum_6 | V (5) | - | - | + | - | + | - | + | - | - | - | - | - | - | - | - | - | - | + | - | - | R |
| *Erigeron glaucus* | Eri.gla_2 | C (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| *Erigeron karvinskianus* | Eri.kar_1 | E (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| | Eri.kar_2 | A (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| | Eri.kar_3 | B (4) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |

| SPECIES [1] | ACCESSION | EXP. (NO. REPS) | MICROSCOPIC EXAMINATION | | | | | | | | | | | | | | | | | VISIBLE EXAMINATION | | | OVERALL SPECIES RESPONSE [2] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | GERMINATION | | | | | PENETRATION | | | COLONISATION | | | | | | | | | REPRODUCTION | | | |
| | | | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | |
| *Erigeron speciosus* | Eri.spe_1 | O (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| *Erigeron speciosus* "Quakeress" | Eri.qua_1 | I (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| | Eri.qua_1 | K (5) | Microscopic development not assessed | | | | | | | | | | | | | | | | | + | - | - | I/HR [4] |
| *Heterotheca grandiflora* | Het.gra_1 | U (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| *Solidago canadensis* | Sol.can_1 | A (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| | Sol.can_3 | B (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| *Symphyotrichum novi-belgii* | Ast.nov_1 | D (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| | Ast.nov_2 | E (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| *Aster cordifolius* | Ast.cor_1 | X (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| *Grindelia camporum* | Gri.cam_1 | AA [5] (5) | Microscopic development not assessed | | | | | | | | | | | | | | | | | + | - | - | I/HR [4] |
| | Gri.cam_1 | AB (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| | Gri.cam_1 | T (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | R |
| *Grindelia robusta* | Gri.rob_2 | X (5) | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| *Eschenbachia leucantha* | Esc.leu_1 | R (5) | - | - | + | - | + | - | - | + | - | - | + | - | - | + | - | - | + | - | + | - | MS |
| | Esc.leu_1 | X (4) | Microscopic development not assessed | | | | | | | | | | | | | | | | | - | + | - | MS |
| *Olearia floribunda* | Ole.flor_1 | B (4) | Microscopic development not assessed | | | | | | | | | | | | | | | | | + | - | - | I/HR [4] |
| *Olearia minor* | Ole.min_1 | C (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| *Olearia phlogopappa* | Ole.phl_1 | E (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| | Ole.phl_2 | F (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| *Vittadinia muelleri* | Vit.mue_1 | F (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| | Vit.mue_1 | R (6) [6] | - | - | + | - | + | - | - | + | - | - | + | - | - | + | + | - | - | + | - | - | HR |
| | Vit.mue_2 | I (4) | - | - | + | - | + | - | - | + | - | - | + | - | - | + | - | - | + | + | - | - | HR |
| | Vit.mue_2 | AA (5) | - | - | + | - | + | - | - | + | - | - | + | - | - | + | - | - | + | + | - | - | HR |
| | Vit.mue_2 | AB (5) | Microscopic development not assessed | | | | | | | | | | | | | | | | | + | - | - | I/HR [4] |

| SPECIES [1] | ACCESSION | EXP. (NO. REPS) | MICROSCOPIC EXAMINATION | | | | | | | | | | | | | | | | | VISIBLE EXAMINATION | | | OVERALL SPECIES RESPONSE [2] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | GERMINATION | | | | | PENETRATION | | | COLONISATION | | | | | | | | | REPRODUCTION | | | |
| | | | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | |
| *Pappochroma bellidioides* | Pap.bel_1 | G (4) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| | Pap.bel_2 | F (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| *Baccharis halimifolia* | Bac.hal_1 | S (5) | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| | Bac.hal_2 | L (5) | - | - | + | - | + | - | - | + | - | - | + | - | + | - | - | - | - | + | - | - | HR |
| | Bac.hal_2 | R (3) [6] | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| *Brachyscome multifida* | Bra.mul_1 | C (4) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| | Bra.mul_2 | D (4) | Microscopic development not assessed | | | | | | | | | | | | | | | | | + | - | - | I/HR [4] |
| *Bellis perennis* | Bel.per_3 | J (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| | Bel.per_5 | O (5) | - | - | + | - | + | - | + | - | - | - | - | - | - | - | - | - | - | + | - | - | HR |
| *Felicia amelloides* | Fel.ame_1 | D (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| | Fel.ame_2 | I (4) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| *Chrysanthemum maximum* | Chr.max_1 | D (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| | Chr.max_2 | J (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| *Glebionis coronaria* | Gle.cor_1 | B (5) | Microscopic development not assessed | | | | | | | | | | | | | | | | | + | - | - | I/HR [4] |
| | Gle.cor_2 | G (5) | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| *Tanacetum vulgare* | Tan.vul_1 | E (5) | Microscopic development not assessed | | | | | | | | | | | | | | | | | + | - | - | I/HR [4] |
| | Tan.vul_2 | G (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| *Cassinia longifolia* | Cas.lon_2 | L (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| | Cas.lon_3 | K (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| *Xerochrysum bracteatum* | Xer.bra_2 | C (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| | Xer.bra_3 | J (5) | - | - | + | - | + | - | + | - | - | - | - | - | - | - | - | - | - | + | - | - | HR |
| *Xerochrysum viscosum* | Xer.vis_1 | D (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| *Ozothamnus diosmifolius* | Ozo.dio_2 | M (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| | Ozo.dio_3 | P (5) | - | - | + | - | + | - | - | + | - | + | - | - | - | - | - | - | - | + | - | - | HR |

| SPECIES [1] | ACCESSION | EXP. (NO. REPS) | MICROSCOPIC EXAMINATION | | | | | | | | | | | | | | | | | VISIBLE EXAMINATION | | | OVERALL SPECIES RESPONSE [2] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | GERMINATION | | | | | PENETRATION | | | COLONISATION | | | | | | | | | REPRODUCTION | | | |
| | | | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | |
| *Ozothamnus secundiflorus* | Ozo.sec_1 | C (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| *Calendula officinalis* | Cal.off_2 | F (5) | - | - | + | - | + | - | - | + | - | + | - | - | - | - | - | - | - | + | - | - | R |
| | Cal.off_2 | AC (5) | - | - | + | - | + | - | - | + | - | + | - | - | - | - | - | - | - | + | - | - | HR |
| | Cal.off_3 | H (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| | Cal.off_3 | AB (5) | Microscopic development not assessed | | | | | | | | | | | | | | | | | + | - | - | I/HR [4] |
| *Dimorphotheca pluvialis* | Dim.plu_1 | F (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| *Dimorphotheca sinuata* | Dim.sin_1 | G (5) | - | - | + | - | + | - | - | + | - | + | - | - | - | - | - | - | - | + | - | - | HR |
| | Dim.sin_3 | M (5) | - | - | + | - | + | - | - | + | - | + | - | - | - | - | - | - | - | + | - | - | HR |
| *Senecio pinnatifolius var. alpinus* | Sen.pin.alp_1 | M (5) | - | - | + | - | + | - | - | + | - | + | - | - | - | - | - | - | - | + | - | - | HR |
| *Senecio pinnatifolius ssp. pinnatifolius* | Sen.pin_2 | E (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| *Abrotanella forsteroides* | Abr.for_1 | O (5), P (5) | Small, cushion-forming alpine plant species, with small, densely imbricate leaves. It was not possible to assess microscopic development. | | | | | | | | | | | | | | | | | + | - | - | I |
| *Bedfordia arborescens* | Bed.arb_1 | T (3) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| *Streptoglossa bubakii* | Str.bub_1 | I (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| | Str.bub_1 | K (5) | Microscopic development not assessed | | | | | | | | | | | | | | | | | + | - | - | I/HR [4] |
| *Centipeda minima* | Cen.min_1 | M (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| | Cen.min_2 | L (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| *Gaillardia grandiflora* | Gai.gra_2 | U (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| | Gai.gra_3 | W (5) | Microscopic development not assessed | | | | | | | | | | | | | | | | | + | - | - | I/HR [4] |
| *Gaillardia pulchella* | Gai.pul_1 | L (5) | - | - | + | - | + | - | + | - | - | - | - | - | - | - | - | - | - | + | - | - | HR |
| *Bidens pilosa* | Bid.pil_1 | H (5) | Microscopic development not assessed | | | | | | | | | | | | | | | | | + | - | - | MR |
| | Bid.pil_1 | S (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | MR |
| | Bid.pil_1 | X (3) | Microscopic development not assessed | | | | | | | | | | | | | | | | | + | - | - | I/HR [4] |

| SPECIES [1] | ACCESSION | EXP. (NO. REPS) | MICROSCOPIC EXAMINATION | | | | | | | | | | | | | | | | | VISIBLE EXAMINATION | | | OVERALL SPECIES RESPONSE [2] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | GERMINATION | | | | | PENETRATION | | | COLONISATION | | | | | | | | | REPRODUCTION | | | |
| | | | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | |
| *Enydra woollsii* | Eny.woo_1 | L (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| | Eny.flu_1 | Q (5) | - | - | + | - | + | - | + | - | - | - | - | - | - | - | - | - | - | + | - | - | HR |
| *Tagetes erecta* | Tag.ere_2 | K (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| *Tagetes patula* | Tag.pat_1 | M (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| | Tag.pat_2 | P (5) | Microscopic development not assessed | | | | | | | | | | | | | | | | | + | - | - | I/HR [4] |
| *Schkuhria pinnata* | Sch.pin_1 | X (3) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| *Helianthus annuus* | Hel.ann_27 | J (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| | Hel.ann_27 | K (3) | Microscopic development not assessed | | | | | | | | | | | | | | | | | + | - | - | I/HR [4] |
| | Hel.ann_28 | H (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| | Hel.ann_6 | N (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| | Hel.ann_3 | AB (5) | - | - | + | - | + | - | - | + | - | + | - | - | - | - | - | - | - | + | - | - | HR |
| *Guizotia abyssinica* | Gui.aby_1 | H (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| | Gui.aby_2 | S (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| *Adenostemma lavenia* | Ade.lav_1 | S (5) | - | - | + | - | + | - | + | - | - | - | - | - | - | - | - | - | - | + | - | - | MR |
| *Ageratina adenophora* | Age.ade_1 | P (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| | Age.ade_2 | O (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| *Arnica chammissonis* | Arn.cha_1 | P (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |
| | Arn.cha_2 | T (5) | - | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | I |

[1] Species listed in the same order as in Table 2.

[2] Response of tested plant species to *Puccinia cnici-oleracei* (ex. *Conyza*) at the end of the experiment. See Table 5 for detail on categories.

[3] Microscopic examination was performed on sample taken during experiment B.

[4] Based on rating in Table 5, without assessment of microscopic development it is not possible to distinguish between Immune and Highly Resistant.

[5] Four leaf pieces instead of three were used to inoculate each plant in the experiments labelled with double letters. In addition, for each plant, the plate with telia used for inoculation was attached to bamboo sticks rather than being fixed to the base of a bucket so that telia were located 12-13 cm above the top-most leaf of the plant.

[6] This species/accession in this experiment was inoculated as outlined in footnote 5.

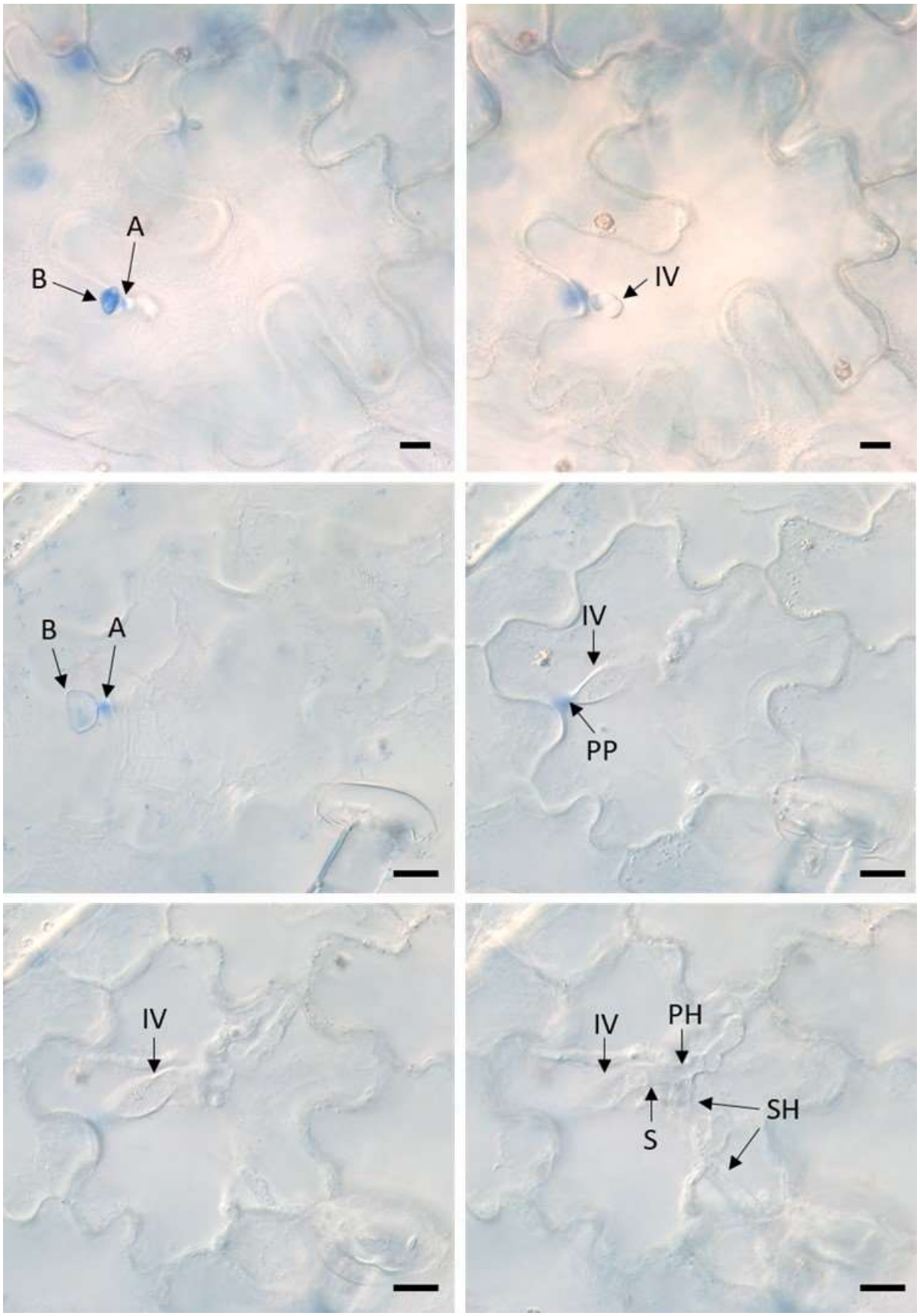


**Figure 11 Initial infection process of *Puccinia cnici-oleracei* (ex*. Conyza*) on leaves of *Conyza bonariensis*. A: Appressorium, B: Basidiospore, IV: Intraepidermal vesicle, PP: Penetration peg, PH: Primary hypha, SH: Secondary hyphae, S: Septum. Micrographs taken at 5 days after inoculation (bars = 10 µm).**

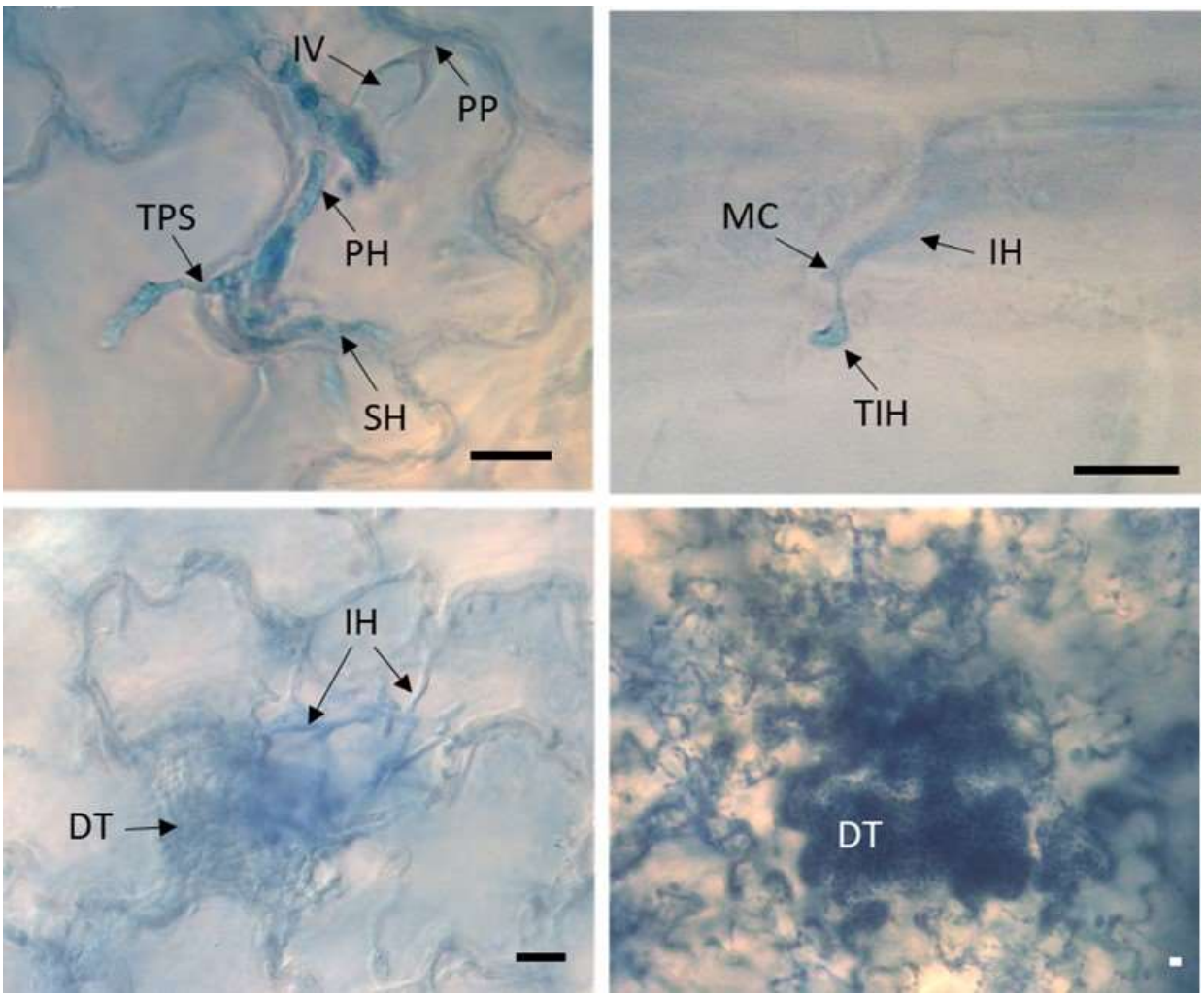


**Figure 12 Advanced infection process of *Puccinia cnici-oleracei* (ex. *Conyza*) on leaves of *Conyza bonariensis*. IV: Intraepidermal vesicle, PP: Penetration peg, PH: Primary hypha, SH: Secondary hyphae, TPS: Transcellular penetration site, MC: Unspecialised mother cell, IH: Intercellular hyphae, TIH: Terminal intracellular hypha, DT: Developing telium. Micrographs taken at 5 or 14 days after inoculation (bars = 10 µm).**

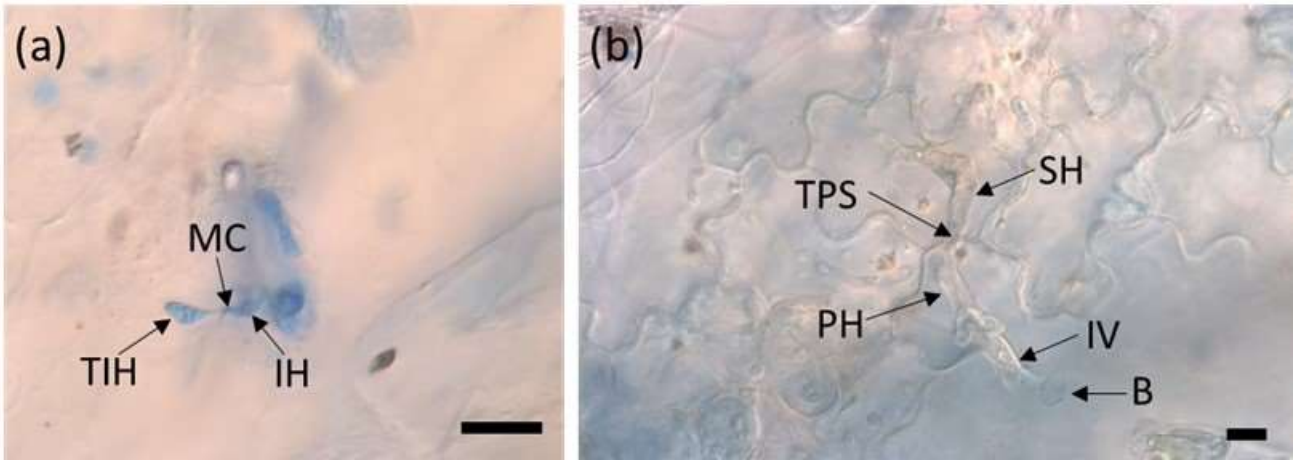


**Figure 13 Normal infection structures of *Puccinia cnici-oleracei* (ex. *Conyza*) observed in leaves of two non-target species. (a) A terminal intracellular hypha in *Vittadinia muelleri* at 5 days after inoculation (DAI). (b) A normal intraepidermal vesicle and primary and secondary hyphae in *Eschenbachia leucantha* at 14 DAI. See Figures 11 and 12 for label legend. (bars = 10 µm).**

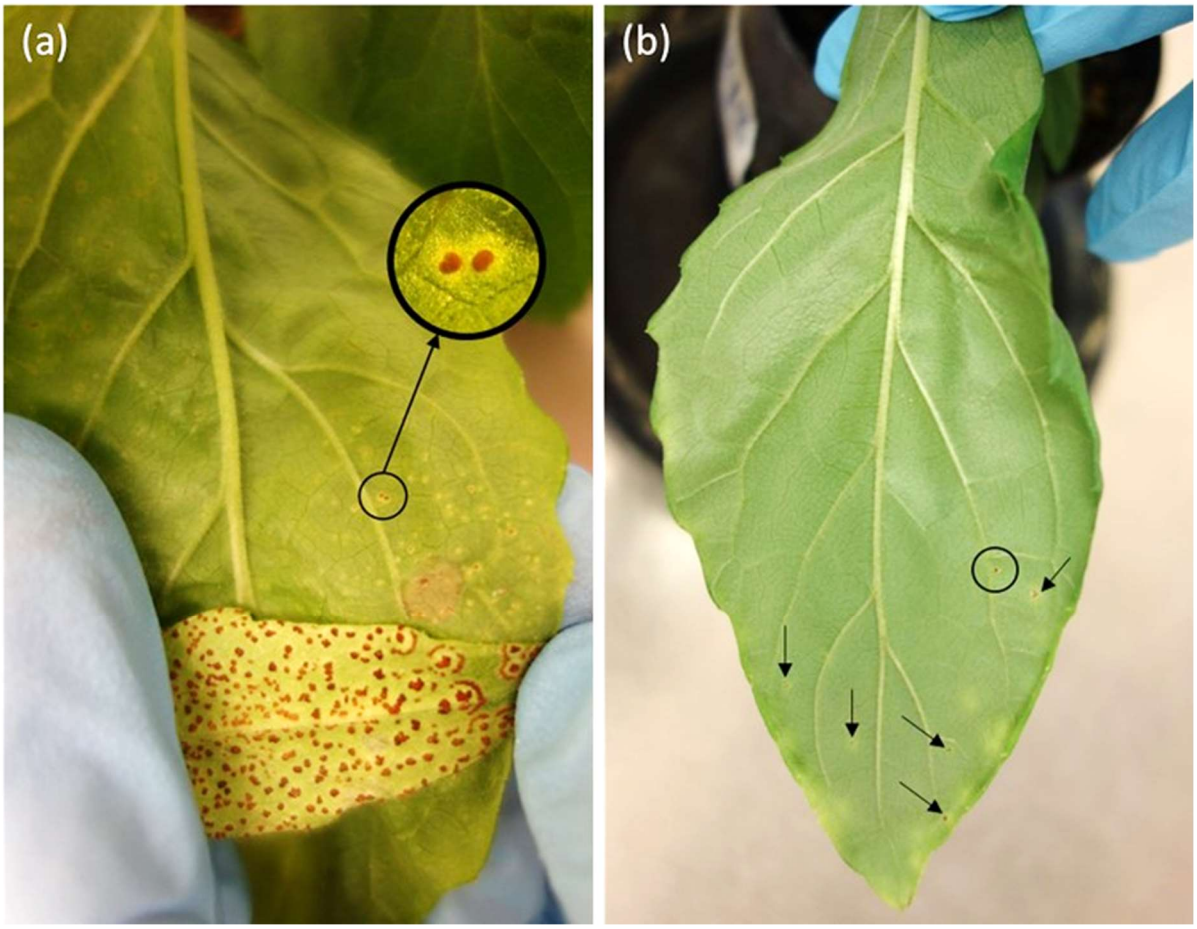


**Figure 14 (a) Normal sized telia observed on *Conyza bonariensis* (small leaf) and chlorotic/necrotic flecks and very few, pin-sized telia (circled) observed on *Eschenbachia leucantha* (large leaf) inoculated with *Puccinia cnici-oleracei* (ex. *Conyza*) in the first experiment at 20 days after inoculation. (b) One minuscule telia (circled) and necrotic flecks (arrows) on one leaf of *E. leucantha* in the second experiment at 7 weeks after inoculation. Development of visible symptoms of *Puccinia cnici-oleracei* (ex. *Conyza*) on tested species**

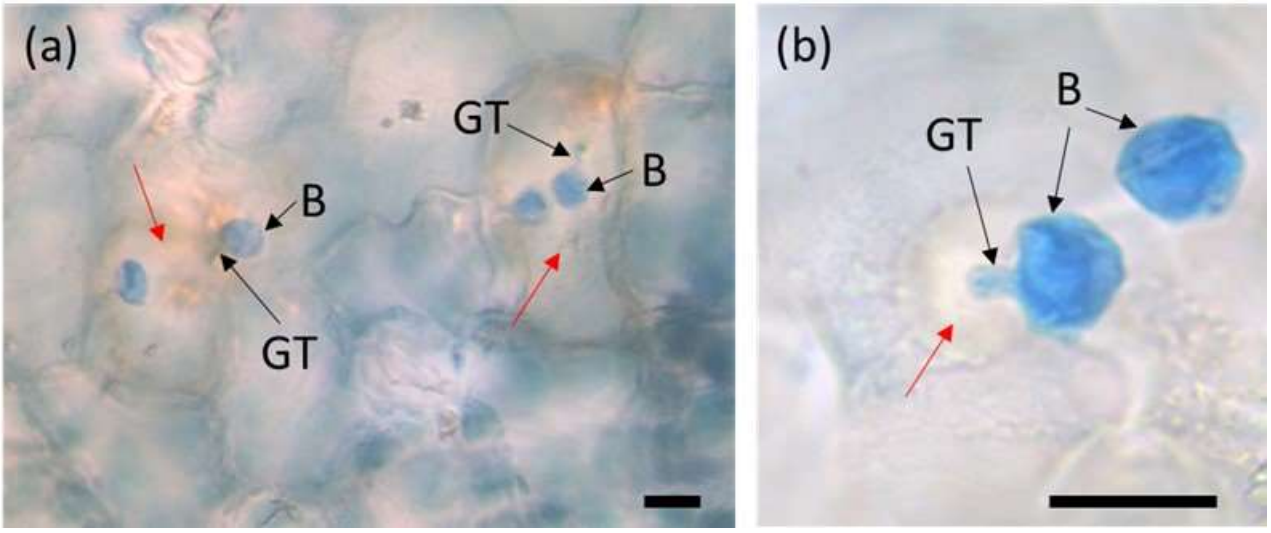


**Figure 15 Germinated basidiospores of *Puccinia cnici-oleracei* (ex. *Conyza*) on *Conyza canadensis* (a) and *Erigeron karvinskianus* (b) without any sign of penetration and associated with defence reactions by the plant cells (red arrows). B: Basidiospore, GT: Germ-tube. (bars = 10 µm).**

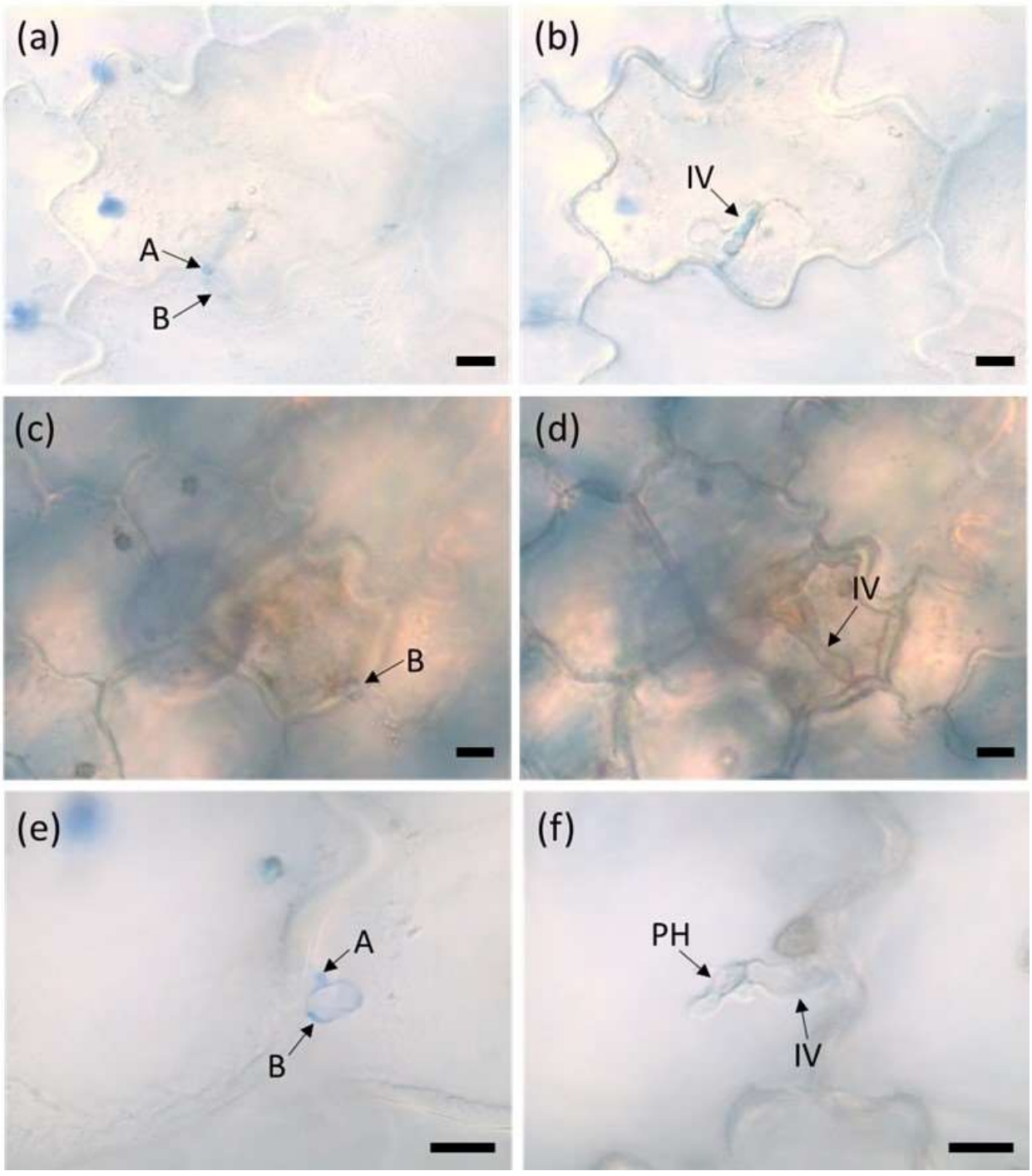


**Figure 16 Abnormal infection structures of *Puccinia cnici-oleracei* (ex. *Conyza*) observed in leaves of different non-target species. Paired micrographs (a-b), (c-d) and (e-f) represent the same field of view taken at different depth. (a-b) Collapsed intraepidermal vesicle in *Xerochrysum bracteatum* at 5 days after inoculation (DAI). (c-d) Dead plant cell associated with a necrotic intraepidermal vesicle in *Conyza sumatrensis* at 35 DAI. (e-f) An intraepidermal vesicle with a very short primary hyphae in *Ozothamnus diosmifolius* at 5 DAI. See Figure 11 for label legend. (bars = 10 µm).**

### 2.12.3.2 Visible symptoms on plant species inoculated with *P. cnici-oleracei* (ex. *Conyza*)

*Puccinia cnici-oleracei* (ex. *Conyza*) developed normal sized telia (> 1 mm diameter) on more than 75% of leaf surface area (rating 6 in Table 4; Fig. 6) on several replicate plants of the nine Australian accessions of *C. bonariensis* tested, including five reported as glyphosate resistant (Table 7). All *C. bonariensis* accessions were categorised as Highly Susceptible (Table 6).

**Table 7 Most severe visible symptoms observed on plant species/accessions inoculated with *Puccinia cnici-oleracei* (ex. *Conyza*). Rating of the symptom, based on Table 4, and percentage of replicates with that rating are presented. Only species/accessions that developed visible symptoms are included.**

| PLANT SPECIES | ACCESSION ID | EXP. | RATING OF MOST SEVERE VISIBLE SYMPTOMS (SEE TABLE 4) | PERCENTAGE OF REPLICATES WITH RATING |
|---|---|---|---|---|
| *Conyza bonariensis* | Con_1 (C2) **(control)** | All | 6 | variable |
| | CB-Goldsworth | E | 6 | 60 |
| | Q.Con.bon_3 | E | 6 | 60 |
| | Con.bon_1 [1] | N | 6 | 60 |
| | Con.bon_6 [1] | N | 6 | 80 |
| | Con.bon_7 [1] | N | 6 | 100 |
| | Con.bon_8 [1] | N | 6 | 60 |
| | Con.bon_9 [1] | N | 6 | 80 |
| | Con.bon_10 [1] | N | 6 | 60 |
| *Conyza sumatrensis* | Con.sum_3 | H | 1 (necrotic blotches) | 100 |
| | Con.sum_4 | F | 1 (necrotic blotches) | 100 |
| | Con.sum (C1, C3) | G | 1 (necrotic blotches) | 40 |
| | | V | 1 (necrotic blotches and chlorotic flecks) | 100 |
| | Con.sum_6 | I | 1 (chlorotic flecks) | 60 |
| | | V | 1 (chlorotic flecks) | 100 |
| *Grindelia camporum* | Gri.cam_1 | T | 1 (chlorotic flecks) | 20 |
| *Eschenbachia leucantha* | Esc.leu_1 | R | 2 | 40 |
| | | X | 2 | 25 |
| *Calendula officinalis* | Cal.off_2 | F | 1 (chlorotic flecks) | 60 |
| *Bidens pilosa* | Bid.pil_1 | H | 1 (necrotic blotches) | 100 |
| | | S | 1 (necrotic blotches) | 100 |
| *Adenostemma lavenia* | Ade.lav_1 | S | 1 (necrotic blotches) | 20 |

[1] Glyphosate resistant accession.

*Conyza sumatrensis*, *Bidens pilosa* and *A. lavenia* developed chlorotic flecks and/or a few necrotic blotches in some replicate plants, while only chlorotic flecks developed on one accession of *C. officinalis* and *Grindelia camporum* (Table 7, Fig. 17). These five species were categorised as Resistant or Moderately Resistant primarily based on the visible symptoms observed (Table 6). In contrast, *E. leucantha* was categorised as Moderately Susceptible based on the chlorotic/necrotic flecks and rare pin-sized telia observed in a few replicates (Fig. 14), combined with microscopic observations (Table 6).

All other non-target plant species tested did not develop any visible symptoms and were rated as either Immune or Highly Resistant based on microscopic examinations of the development of the fungus on these species.

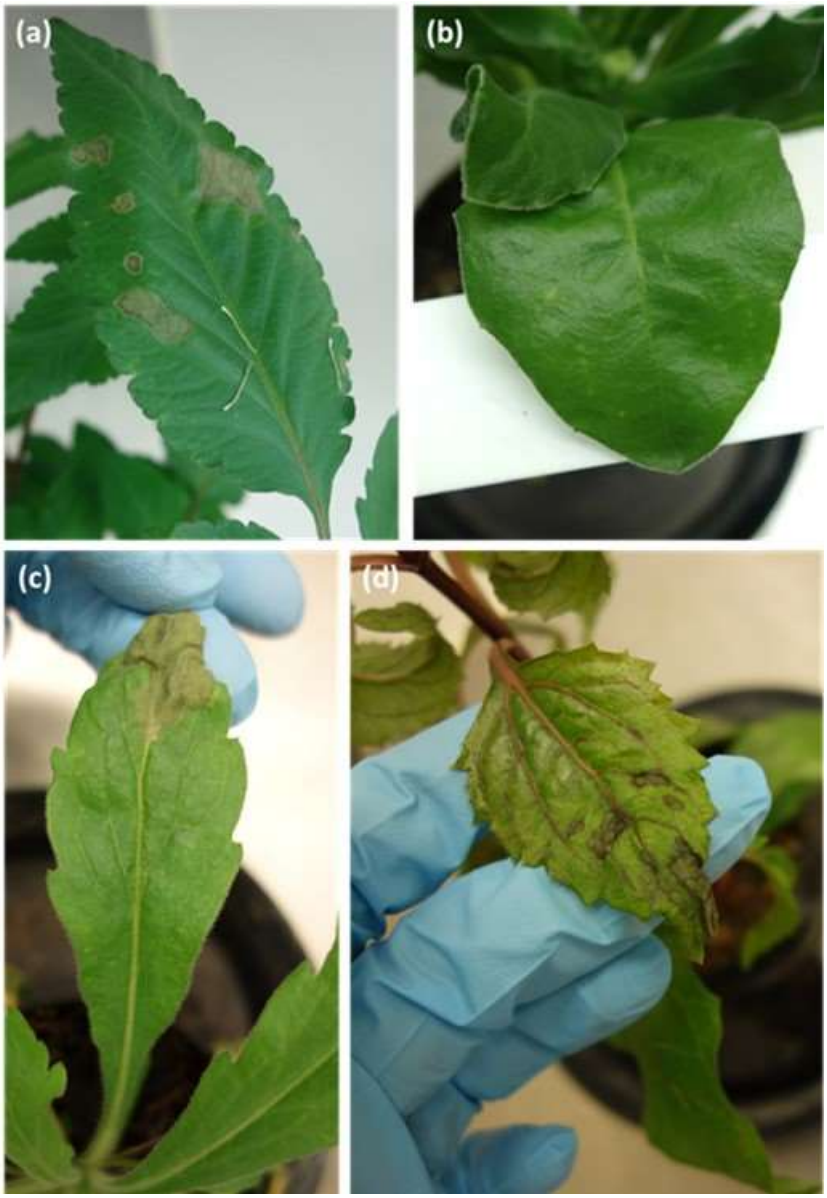


**Figure 17 Responses of non-target plants to *Puccinia cnici-oleracei* (ex. *Conyza*). (a) Necrotic blotches on *Bidens pilosa* and (b) chlorotic flecks on *Calendula officinalis* at 28 days after inoculation (DAI). (c) Necrotic blotch and chlorotic flecks on *Conyza sumatrensis* at 14 DAI. (d) Necrotic blotches on *Adenostemma lavenia* at 28 DAI.**

# 3 Discussion

Several microcyclic rust fungi with the same basic morphology, that are specialised on different plant species in the Asteraceae family, are known from both the Old and New Worlds. On one hand, the name *P. cnici-oleracei* in a broad sense is used by some contemporary authors to refer to this cosmopolitan complex of rust fungi (Hennen et al. 2005, Berndt 2018). On the other hand, the various names previously created for these similar rust fungi with different hosts are still used in the literature and databases such as GenBank. This taxonomic imbroglio requires comprehensive investigation, including sequencing of several gene regions across many rust accessions from different hosts, to either justify the use of a single name for this complex or different names for each of the fungi based on their host range. Extensive sequencing could also reveal the existence of two rust morphospecies complexes based on geographical origin (American or Eurasian) (Seier et al. 2009). While *P. cnici-oleracei* sensu lato is the name used to refer to this single putative complex, Hennen (pers. comm. 2007 cited in Seier et al. 2009) proposed that *P. xanthii*, which is an older published name, would be more appropriate to use.

We decided to use the name *P. cnici-oleracei* (ex. *Conyza*) for the microcyclic rust fungus found on *Conyza* sp. in Colombia based on the: (i) above background information, (ii) phylogenetic relationships of its ITS sequence with that of other species, and (iii) results from a cross-inoculation experiment with a similar rust fungus found on *E. sonchifolia* at the same site. The addition of 'ex. *Conyza*' between parentheses after the name was made to indicate that the accession of *P. cnici-oleracei* used in the research originates from *Conyza* sp. and thus avoid possible confusion.

Results from the series of host-specificity experiments conducted with *P. cnici-oleracei* (ex. *Conyza*) in the biosecurity containment facility demonstrated its high level of specialisation on the target weed *C. bonariensis*. The fungus caused disease symptoms on all *C. bonariensis* accessions from Australia tested, but not on any of the other species in the same subtribe (Conyzinae) included in the experiments. While the introduced and naturalised *Conyza sumatrensis* and *Conyza canadensis* that are common in Australia could be sourced and tested, we failed to obtain propagation material of the less common, introduced *Conyza bilbaoana* and *Conyza primifolia*. We made several attempts to source *Erigeron conyzoides*, the sole native species in this genus that occurs in Australia. We approached and sought help from staff of many herbaria and seed collections and individuals from various organisations regularly performing vegetation surveys in areas where the species has been previously recorded. Plants of *E. conyzoides* were not encountered in any of these field surveys, but we were able to obtain seed from old herbarium samples kept at the Royal Botanic Gardens Victoria. Unfortunately, none of these seeds germinated and it was not possible to propagate the species for inclusion in host-specificity experiments.

The isolate of *P. cnici-oleracei* (ex. *Conyza*) used in the experiments originated from a plant in Colombia molecularly identified as *C. sumatrensis* using markers developed by Wang et al. (2018) with material from Australia. It was thus surprising that the different Australian accessions of *C. sumatrensis* tested only developed chlorotic flecks and necrotic blotches, and

no telia following inoculation with the rust isolate. This highlighted that the plant from which the rust isolate is derived from in Colombia is different to the Australian *C. sumatrensis* accessions used in our experiments, and may indeed be a different species, despite the molecular markers indicating that they are the same species. Similar flecks and blotches following inoculation with the rust fungus were also the only visible symptoms observed in some plants of other species tested (*G. camporum*, *C. officinalis*, *B. pilosa*, *A. lavenia*), which belong to different tribes in the Asteroideae sub-family. The chlorotic flecks most likely corresponded to hypersensitive responses by the plant to the fungus attempting to penetrate the leaf (Balint-Kurti 2019). The necrotic blotches observed could have been the result of numerous basidiospores landing and trying to penetrate epidermal cells in the same area of a leaf, thus triggering an extensive defence reaction by the plant. Alternatively, some of the plants may have been especially sensitive to the long (48 h) exposure to a high humidity environment during the inoculation period and develop some necrosis.

Although *P. cnici-oleracei* (ex. *Conyza*) developed the same infection structures in leaves of *C. bonariensis, E. leucantha* and *V. muelleri*, colonisation of plant tissue by intercellular hyphae was restricted in the latter two species. The development of terminal intracellular hyphae (like haustoria in macrocyclic rust fungi) in *V. muelleri* and the absence of any visible symptoms indicated that the resistance mechanisms in this species operate at the level of these fungal feeding structures within plant cells (Garnica et al. 2014). In contrast, for all other species tested, plants deployed defence mechanisms and stopped infection in the early stage of penetration attempts by the fungus or very soon after it developed an intraepidermal vesicle and in a few instances a primary hypha.

*Eschenbachia leucantha*, the representative species in the subtribe Grangeinae tested, was the only species on which *P. cnici-oleracei* (ex. *Conyza*) proceeded to develop telia, albeit minuscule and infrequently, amongst the many chlorotic and necrotic flecks that developed. These pin-sized telia only developed in one or two replicate plants in each experiment, and only one of the telia used to subsequently inoculate *C. bonariensis* resulted in infection and development of a few telia. We chose the highly susceptible *C. bonariensis* for these inoculations with the small telia from *E. leucantha* in order to increase chances of infection and telia development occurring. However, it is highly unlikely that *P. cnici-oleracei* (ex. *Conyza*) would be able to complete its life cycle on *E. leucantha* in the laboratory or the field considering its low level of susceptibility. It is noteworthy that *E. leucantha* was previously known as *Conyza leucantha* (Chen et al. 2011). Brouillet et al. (2009) determined that African and Asian species previously assigned to *Conyza* do not belong to this North American taxon, but to the unrelated subtribe Grangeinae of the Astereae. Most native species of China previously treated under *Conyza*, including *C. leucantha*, were thus assigned to the genus *Eschenbachia* (Chen et al. 2011).

Based on results from host-specificity experiments, the level of risk to non-target plant species associated with releasing *P. cnici-oleracei (ex. Conyza)* in Australia is considered negligible, and permission for its release from the biosecurity containment facility is thus sought. Providing environmental conditions are conducive for disease development, we expect *P. cnici-oleracei* (ex. *Conyza*) to cause recurrent and severe infections on *C. bonariensis* over each growing season.

The repeated weekly inoculations experiment that we performed with *P. cnici-oleracei* (ex. *Conyza*) in the biosecurity containment facility, showed how debilitating the fungus can be for *C. bonariensis* growth and reproduction. Severely diseased *C. bonariensis* in the field should produce less flowers and seeds, which should in turn reduce invasion of fallows, cultivated fields and other habitats by the weed. A related microcyclic rust fungus, *P. xanthii*, which infects species in the Noogoora burr complex, has had adverse impacts on its hosts, especially in coastal areas where more rainfall occurs. Since its first record in Australia in 1975 (Alcorn 1975), *P. xanthii* has caused a gradual and significant reduction in Noogoora burr infestations in south-east Queensland (Morin et al. 1996). The fungus, however, has not been as damaging on the weed in drier inland regions. Similarly, dry summer conditions in Queensland in the years following the deliberate release of *P. xanthii* var *parthenii-hysterophorae* in 2000 resulted in low levels of disease and a negligible impact on the target weed *P. hysterophorus* (Dhileepan et al. 2006). The initial release strategy for *P. cnici-oleracei* (ex. *Conyza*) will need to focus on the most climatically suitable areas to ensure the fungus readily establishes and builds up its population.

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Any of these references can be provided in electronic form if requested. Contact Louise Morin (louise.morin@csiro.au; Ph: (02) 6246 4355)


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# Appendix A Phylogenetic relatedness of tribes within the Asteroideae subfamily.

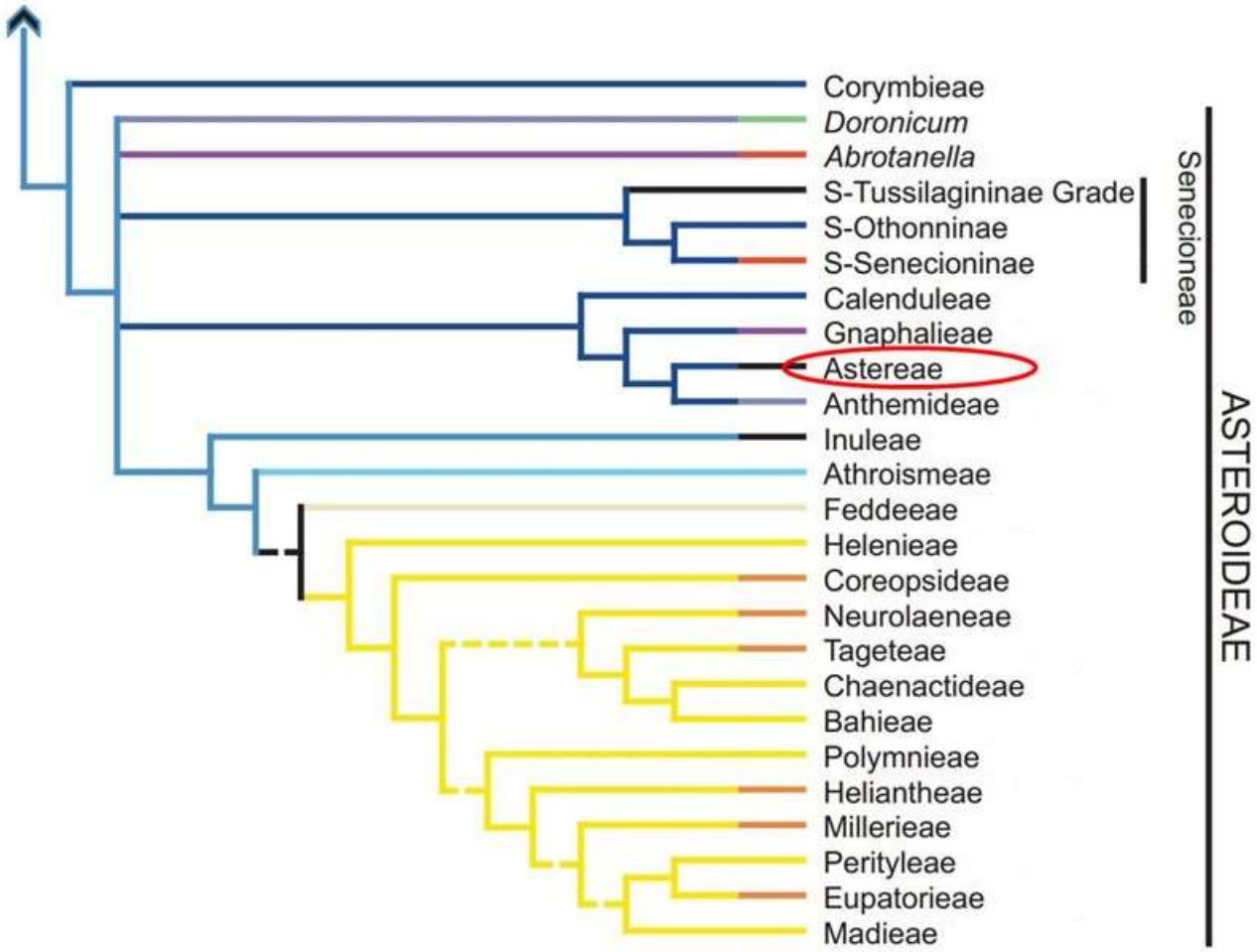


**Figure A-1 A portion of a summary metatree for Asteraceae, as depicted in Funk et al. (2009), indicating the phylogenetic relationship of tribes within the Asteraceae subfamily Asteroideae. The phylogenetic position of the tribe Astereae, to which the genus *Conyza* belongs, is circled for reference.**

# Appendix B Attempts to source seeds or plants of *Erigeron conyzoides*

**Responses from the various individuals in different organisations we approached in 2019 in our attempts to source seeds or plants of *Erigeron conyzoides*. Note that the names of individuals contacted have been withheld for privacy reasons.**

| ORGANISATION | STATE | COMMENT |
|---|---|---|
| Royal Botanic Gardens, Victoria Herbarium | VIC | • Kept an eye out for the species during field surveys in the region where it has been recorded before, but it was not found.<br>• Extracted seeds from herbarium vouchers and sent to us. None germinated. |
| Australian National Herbarium | ACT | Checked five herbarium specimens of the species, but none with seeds. |
| Upper Snowy Landcare Network | NSW | Kept an eye out for the species during field surveys in the region where it has been recorded before, but it was not found. |
| CSIRO | NSW | Kept an eye out for the species during field surveys in the region where it has been recorded before, but it was not found. |
| Australian Botanic Garden, Mount Annan | NSW | • Kept an eye out for the species during field surveys in the region where it has been recorded before, but it was not found.<br>• Species not in their seed collections. |
| La Trobe University | VIC | Kept an eye out for the species during field surveys in the region where it has been recorded before, but it was not found. |
| Office of Environment and Heritage (South East Branch) | NSW | Kept an eye out for the species during field surveys in the region where it has been recorded before, but it was not found. |
| Office of Environment and Heritage (Sydney) | NSW | Kept an eye out for the species during field surveys in the region where it has been recorded before, but it was not found. |
| Australian National Botanic Gardens | ACT | Species added to their collection priority list, but it was not found. |

# Appendix C Source of accessions of each non-target plant species tested

| PLANT SPECIES | ACCESSION ID | SOURCE | MATERIAL | LOCATION OR PROVIDER NAME | STATE |
|---|---|---|---|---|---|
| *Conyza bonariensis* | Con_1 (C2) | Field | Seed | Abercrombie Road, Tuena | NSW |
| | CB-Goldsworth | Field | Seed | Biala | NSW |
| | Q.Con.bon_3 | Field | Seed | Chapel Hill, Carinya St, Brisbane | QLD |
| | Con.bon_1 | Field | Seed | Brisbane | QLD |
| | Con.bon_6 | Field | Seed | Blaxland (Glyphosate resistant) | QLD |
| | Con.bon_7 | Field | Seed | Byee (Glyphosate resistant) | QLD |
| | Con.bon_8 | Field | Seed | Euthulla (Glyphosate resistant) | QLD |
| | Con.bon_9 | Field | Seed | Goondiwindi (Glyphosate resistant) | QLD |
| | Con.bon_10 | Field | Seed | Bundemar (Glyphosate resistant) | NSW |
| *Conyza canadensis* | Con.can_1 | Field | Seed | Wagga Wagga | NSW |
| *Conyza sumatrensis* | Con.sum_3 | Field | Seed | Esk | QLD |
| | Con.sum_4 | Field | Seed | Beaudesert Road, Marooka | QLD |
| | Con.sum (C1, C3) | Field | Seed | Abercrombie Road, Tuena | NSW |
| | Con.sum_6 | Field | Seed | Tilba | NSW |
| *Erigeron karvinskianus* | Eri.kar_1 | Commercial | Plants | Russ Australian Natives | QLD |
| | Eri.kar_2 | Field | Plants | Wantalanya Chalets Garden | QLD |
| | Eri.kar_3 | Commercial | Plants | Plants Management Australia | ACT |
| *Erigeron glaucus* | Eri.gla_2 | Commercial | Plants | Lambley Nursery | VIC |
| *Erigeron speciosus* | Eri.spe_1 | Commercial | Seed | Seedscape | VIC |
| *Erigeron speciosus* "Quakeress" | Eri.qua_1 | Commercial | Plants | Greenpatch Organic Seeds & Plants | NSW |
| *Heterotheca grandiflora* | Het.gra_1 | Field | Seed | Shoal Bay | NSW |
| *Solidago canadensis* | Sol.can_1 | Commercial | Plants | Nutshell Nursery | NSW |
| | Sol.can_3 | Commercial | Plants | Nutshell Nursery | NSW |
| *Symphyotrichum novi-belgii* | Ast.nov_1 | Commercial | Seed | Southern Harvest | TAS |
| | Ast.nov_2 | Commercial | Plants | Planters Patch | NSW |
| *Aster cordifolius* | Ast.cor_1 | Commercial | Plants | Frogmore Gardens | VIC |
| *Grindelia camporum* | Gri.cam_1 | Commercial | Plants | Mudbrick Cottage Herbfarm | QLD |
| *Grindelia robusta* | Gri.rob_1 | Commercial | Seed | Eden Seed | QLD |
| *Eschenbachia leucantha* | Esc.leu_1 | Field | Seed | Smithfield Conservation Park | QLD |

| PLANT SPECIES | ACCESSION ID | SOURCE | MATERIAL | LOCATION OR PROVIDER NAME | STATE |
|---|---|---|---|---|---|
| *Olearia phlogopappa* | Ole.phl_1 | Commercial | Plants | Cool Country Natives | ACT |
| | Ole.phl_2 | Commercial | Plants | Australian National Botanic Gardens | ACT |
| *Olearia minor* | Ole.min_1 | Commercial | Plants | Cool Country Natives | ACT |
| *Olearia floribunda* | Ole.flo_1 | Commercial | Plants | Australian National Botanic Gardens | ACT |
| *Vittadinia muelleri* | Vit.mue_1 | Field | Seed | Bruce Ridge Nature Reserve | ACT |
| | Vit.mue_2 | Commercial | Plants | Plants of Tasmania Nursery | TAS |
| *Pappochroma bellidioides* | Pap.bel_1 | Field | Seed | Kosciuszko National Park | NSW |
| | Pap.bel_2 | Field | Seed | Kosciuszko National Park | NSW |
| *Baccharis halimifolia* | Bac.hal_1 | Field | Seed/cuttings | Fig Tree Pocket | QLD |
| | Bac.hal_2 | Field | Seed | Carbrook Wetlands Conversation Park | QLD |
| *Brachyscome multifida* | Bra.mul_1 | Commercial | Plants | Australian National Botanic Gardens | ACT |
| | Bra.mul_2 | Commercial | Plants | Australian National Botanic Gardens | ACT |
| *Bellis perennis* | Bel.per_3 | Commercial | Seed | Eden Seed | QLD |
| | Bel.per_5 | Commercial | Plants | Greengold Nursery Federation Square | ACT |
| *Felicia amelloides* | Fel.ame_1 | Commercial | Plants | Brenlissa Online Nursery | VIC |
| | Fel.ame_2 | Commercial | Plants | Garden Express | VIC |
| *Chrysanthemum maximum* | Chr.max_1 | Commercial | Seed | Southern Harvest | TAS |
| | Chr.max_2 | Commercial | Seed | Eden Seed | QLD |
| *Glebionis coronaria* | Gleb.cor_1 | Commercial | Seed | Royston Petrie Seeds | NSW |
| | Gleb.cor_2 | Commercial | Seed | The Seed Collection | VIC |
| *Tanacetum vulgare* | Tan.vul_1 | Commercial | Seed | The Diggers Club | NSW |
| | Tan.vul_2 | Commercial | Seed | The Seed Collection | VIC |
| *Cassinia longifolia* | Cas.lon_2 | Commercial | Plants | Australian National Botanic Gardens | ACT |
| | Cas.lon_3 | Commercial | Plants | Cool Country Natives | ACT |
| *Xerochrysum bracteatum* | Xer.bra_2 | Commercial | Seed | Australian National Botanic Gardens | ACT |
| | Xer.bra_3 | Commercial | Seed | Eden Seed | QLD |
| *Xerochrysum viscosum* | Xer.vis_1 | Commercial | Seed | Black Mountain | ACT |
| *Ozothamnus diosmifolius* | Ozo.dio_2 | Commercial | Plants | Cool Country Natives | ACT |
| | Ozo.dio_3 | Commercial | Seed | Australian Seed | WA |
| *Ozothamnus secundiflorus* | Ozo.sec_1 | Commercial | Plants | Cool Country Natives | ACT |
| *Calendula officinalis* | Cal.off_2 | Commercial | Seed | Southern Harvest | TAS |
| | Cal.off_3 | Commercial | Seed | Eden Seed | QLD |
| *Dimorphotheca pluvialis* | Dim.plu_1 | Commercial | Seed | B and T World Seeds | France |
| *Dimorphotheca sinuata* | Dim.sin_1 | Commercial | Seed | B and T World Seeds | France |

| PLANT SPECIES | ACCESSION ID | SOURCE | MATERIAL | LOCATION OR PROVIDER NAME | STATE |
|---|---|---|---|---|---|
| | Dim.sin_3 | Commercial | Seed | Australian Seed | WA |
| *Senecio pinnatifolius* ssp. *pinnatifolius* | Sen.pin_1 | Field | Seed | Hankercheif Beach | NSW |
| *Senecio pinnatifolius* var. *alpinus* | Sen.pin.alp_1 | Field | Seed | Kosciuszko National Park | NSW |
| *Abrotanella forsteroides* | Abr.for_1 | Commercial | Plants | Plants of Tasmania Nursery | TAS |
| *Bedfordia arborescens* | Bed.arb_1 | Commercial | Seed | Seeds of Gippsland | VIC |
| *Streptoglossa bubakii* | Str.bub_1 | Commercial | Seed | Nindethana Seed Service | WA |
| *Centipeda minima* | Cen.min_1 | Field | Seed | Kununurra | WA |
| | Cen.min_2 | Field | Seed | Warrabah National Park | NSW |
| *Gaillardia grandiflora* | Gai.gra_2 | Commercial | Seed | The Seed Collection | VIC |
| | Gai.gra_3 | Commercial | Seed | Mr Fothergill's | ACT |
| *Gaillardia pulchella* | Gai.pul_1 | Commercial | Seed | Ole Lantana's Seed Store | QLD |
| *Bidens pilosa* | Bid.pil_1 | Field | Seed | Mareeba | QLD |
| *Enydra woollsii* | Eny.woo_1 | Field | Cuttings | Cattai Wetland | NSW |
| | Eny.flu_1 | Field | Seed | Great Lakes area | NSW |
| *Tagetes erecta* | Tag.ere_1 | Commercial | Seed | D.T. Brown seeds | |
| *Tagetes patula* | Tag.pat_1 | Commercial | Seed | Mr Fothergill's Seeds | NSW |
| | Tag.pat_2 | Commercial | Seed | Southern Harvest | TAS |
| *Schkuhria pinnata* | Sch.pin_1 | Field | Seed | Tamworth | NSW |
| *Helianthus annuus* | | | | | |
| Cultivar 'Giant Single' | Hel.ann_27 | Commercial | Seed | The Seed Collection | VIC |
| Cultivar 'Sunbird' | Hel.ann_28 | Commercial | Seed | The Seed Collection | VIC |
| Cultivar 'Hyoleic 41' | Hel.ann_6 | Commercial | Seed | Elite Pacific Seeds | QLD |
| Cultivar 'Ausi-Gold 62' | Hel.ann_3 | Commercial | Seed | Lefroy Seeds | VIC |
| *Guizotia abyssinica* | Gui.aby_1 | Commercial | Seed | Australian Temperature Field Crops Collection | |
| | Gui.aby_2 | Commercial | Seed | Fairdinkum Seeds | QLD |
| *Adenostemma lavenia* | Ade.lav_1 | Field | Seed | Toomba Station | QLD |
| *Ageratina adenophora* | Age.ade_1 | Field | Seed | Blue Mountains | NSW |
| | Age.ade_2 | Field | Seed | Windy Gully, Wollongong | NSW |
| *Arnica chammissonis* | Arn.cha_2 | Commercial | Plants | Mudbrick Cottage Herbfarm | QLD |
| | Arn.cha_3 | Commercial | Plants | All Rare Herbs | QLD |

[1] Tribes of the subfamily Asteroideae of the family Asteraceae.

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**Contact us**

1300 363 400
+61 3 9545 2176
csiroenquiries@csiro.au
csiro.au

**For further information**

**Health and Biosecurity**
Dr Louise Morin
+61 2 6246 4355
louise.morin@csiro.au