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Information package to support application to release the rust fungus *Baeodromus eupatorii* for the biological control of crofton weed (*Ageratina adenophora*) in Australia

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Copyright and disclaimer

Important disclaimer

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Executive summary

Prior to 1984, when the *Biological Control Act* was enacted, crofton weed (*Ageratina adenophora*; Asteraceae) was a target for biological control in Australia. Surveys of natural enemies of crofton weed in Mexico, the region of origin of this weed, undertaken by South African colleagues in 2008, identified the rust fungus *Baeodromus eupatorii* as a potential biological control candidate agent. The rust was imported into the QC3 Microbiological area of the CSIRO Black Mountain Containment Facility in Canberra in December 2011. Once a culture of the fungus was established, a series of tests were performed to investigate its host-range. The selection of the 60 non-target plant species for testing was based on a recent molecular phylogeny of tribes in the family Asteraceae. Each species was tested in two separate trials (unless otherwise indicated; five replicates per species per trial) and crofton weed plants were used as positive controls. Results demonstrated that *B. eupatorii* is a highly host specific rust fungus, being capable of successfully developing only on three species within the *Ageratina* genus (*A. adenophora*–crofton weed, *A. altissima*, *A. riparia*; all introduced non-desirable species). Across all experiments, both crofton weed and *A. altissima* consistently supported development of abundant reproductive structures (pycnia and telia), which produced basidiospores that were capable of infecting either species, demonstrating that the rust can complete its life cycle on these hosts. In contrast, *A. riparia* was not as suitable a host for *B. eupatorii* as these other two species. While the rust produced abundant pycnia on a few very young, still expanding leaves of *A. riparia* when a very high density of inoculum was used, in the standard host-specificity trials pycnia were infrequently produced and telia were often associated with necrosis. All other 58 non-target plant species tested, which included a large number of other representatives from the Eupatorieae tribe (including the two Australian native *Adenostemma* species) and representatives from across tribes related to Eupatorieae within the sub-family Asteroideae of the Asteraceae family that are present in Australia, were either rated as immune or highly resistant to the rust. The possible infection of *A. riparia* in the field, should *B. eupatorii* be released in Australia, would not pose a problem since this species is an undesirable environmental weed. Damage on *A. altissima* (syn *Eupatorium rugosum*) is most likely to occur in the field, but as far as we know this species is not widely grown in gardens in Australia and it has been assessed by DAFF Biosecurity as posing a high risk of becoming a weed in Australia. We conclude that the level of risk associated with releasing *B. eupatorii* is acceptable and that it will be a potentially effective biological control agent. We seek permission for its release in Australia.

Information on biological control agent

Agent name

Order: Uredinales

Family: *Pucciniosiraceae*

Genus: *Baeodromus*

Species: *eupatorii* (Arthur) Arthur 1907

Common Name: Crofton weed rust

Voucher specimen: A voucher herbarium specimen will be deposited in the Plant Pathology Herbarium of NSW Department of Agriculture, Orange, as soon as permission is granted to release the rust in Australia.

Brief biology of the agent

Baeodromus eupatorii was described in 1907 from the host *Ageratina pazcuarensis* (syn. *Eupatorium pazcuarense*) near Amecameca in Mexico (Buriticá and Hennen 1980). It is a microcyclic and autoecious rust (no alternate hosts) with only pycnia (spermogonia) and telia reported to be produced on *Eupatorium* or *Ageratina* species.

The rust produces numerous golden-orange telia (0.2-0.3 mm diam) in circular groups (1-4 mm diam, but sometimes up to 10 mm), often encircling small pycnia (100-150 µm diam), on young leaves and stems (Buriticá and Hennen 1980). Pycnia (Fig. 1A, B) are mostly produced in the upper surface of leaves, and telia on the under surface (Fig. 1C, D). Petioles and stems with pycnia and telia are often swollen and contorted (Fig. 1E, F). Cross-fertilisation between pycnia is necessary for telia to develop. This typically occurs under natural conditions by insects transferring pycniospores produced in sweet, attractive mucus between pycnia. Cross-fertilisation between pycnia under laboratory conditions can be performed using a fine hair brush.

Teliospores are 1-celled, 16-32 × 13-21 µm, mostly ellipsoid and without a differentiated germ pore (Buriticá and Hennen 1980). They are strongly attached to the telium by their pedicels and are not individually wind-borne. They are capable of germination immediately upon formation and produce 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, pycnia with visible mucus containing pycniospores are observed. Providing cross-fertilisation occurred, telia begin to develop within the next few days.

Native range of the agent

Baeodromus eupatorii has only been recorded from Central America (Mexico, Guatemala and Honduras) (Buriticá and Hennen 1980, Farr et al. 2013). It has never been recorded in Australia or anywhere else outside its native range.

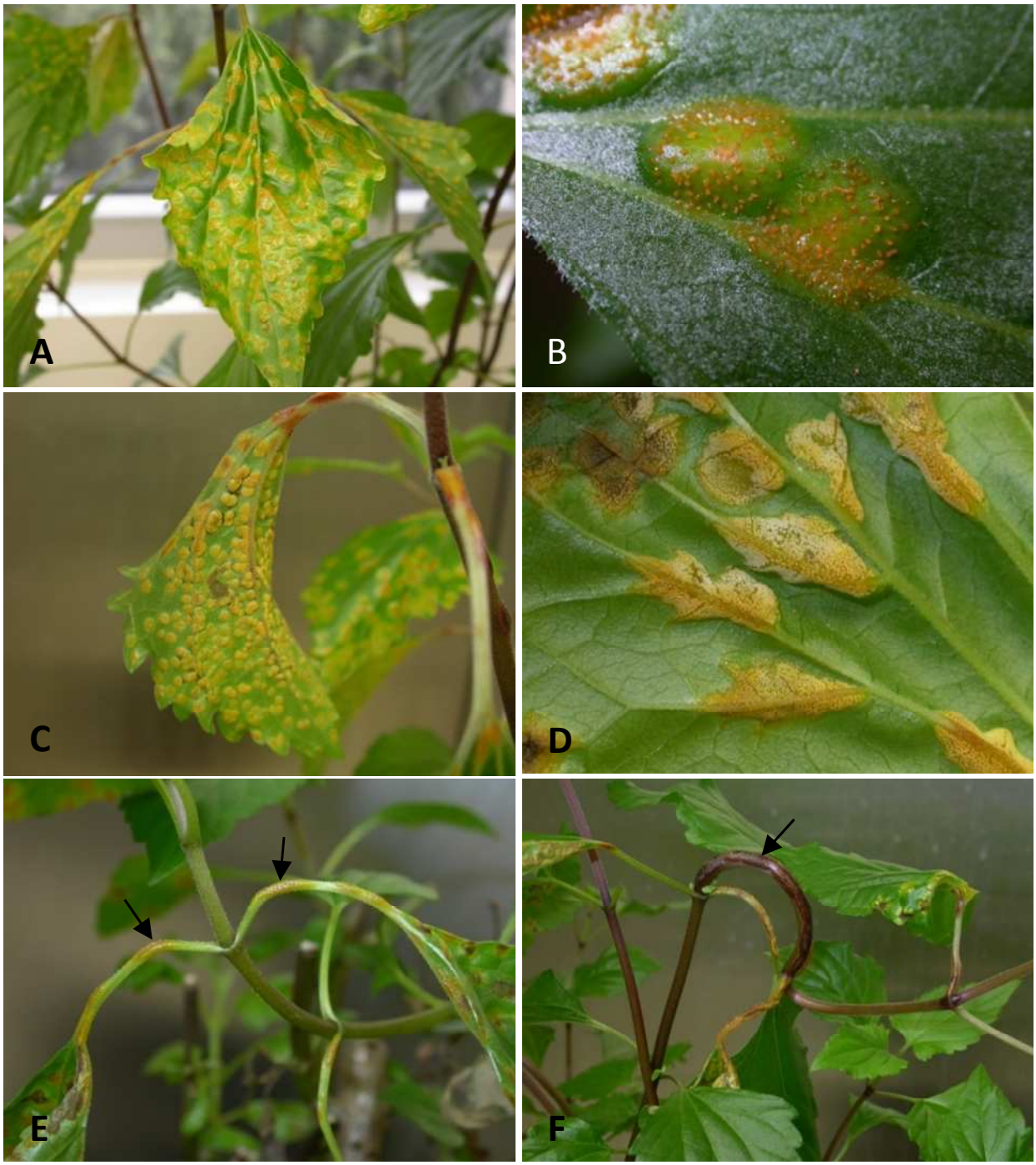

Figure 1 Disease symptoms caused by *Baeodromus eupatorii* on crofton weed (*Ageratina adenophora*). A. Sori of pycnia on the upper surface of a leaf. B. Close-up of pycnia. C. Sori of telia on the under surface of a leaf. D. Close-up of telia. E. Sori of pycnia and telia on leaf petioles (arrows). F. Contorted stem (arrow) due to development of sori.

Related species to the agent and a summary of their host range

The following rust fungi have been listed in Arthur (1905), Holway (1918) and Buriticá and Hennen (1980) for the genus *Eupatorium* in Central and South America: *Cionothrix basicrassa*, *Cionothrix praelonga*, *Coleosporium eupatorii*, *Chardoniella andina*, *Chardoniella capitata*, *Chardoniella gynoxides*, *Puccinia inanipes*, *Puccinia conoclinii*, *Puccinia espinosara*, *Puccinia rosea*, *Pucciniosira arthuri*, *Pucciniosira cumminsiana*.

Only two rust fungi, *B. eupatorii* and *P. conoclinii* have been recorded from crofton weed (*Ageratina adenophora*) (Farr et al. 2013). Based on herbarium records, *B. eupatorii* has also been recorded in Mexico, Guatemala and Honduras on a few other *Ageratina* and *Eupatorium* species (*A. pichinchensis* (syn. *A. aschenborniana* and *E. aschenbornianum)*, *A. mairetiana* (syn. *E. mairetianum*), *A. pazcuarensis* (syn. *E. pazcuarense*), and *E. pycnocephalum* (Buriticá and Hennen 1980, Farr et al. 2013). *Puccinia conoclinii* has only been recorded on *Eupatorium glandulosum*, a synonym of *A. adenophora*, in Guatemala (Arthur 1918, Farr et al. 2013).

Only seven species of *Baeodromus* have been described (Table 1). Most of the known host plants of members of this fungal genus occur in the Asteraceae family.

Table 1 Species of *Baeodromus* that have been described.

BAEODROMUS SPECIES	PLANT HOSTS
B. albertensis	*Senecio eremophilus*
B. californicus	*Senecio douglasii*, *Senecio* sp.
B. eupatorii	*Ageratina adenophora* (syn. *Eupatorium adenophorum*), *Adenophora pichinchensis* (syn. *Eupatorium aschenbornianum*), *Adenophora mairetiana* (syn. *Eupatorium mairetianum)*, *Adenophora pazcuarensis* (syn. *Eupatorium pazcuarense*), *Eupatorium pycnocephalum*, *Eupatorium* sp.
B. holwayi	*Senecio argutus*, *Senecio cinerarioides*, *Senecio nerarioides*, *Senecio warszewiczii*
B. ranunculi	*Ranunculus flagelliformis*
B. senecionis	*Senecio betonicaefolius*, *Senecio* sp.
B. tranzschelii	*Urtica laetevirens*

Source: Farr *et al.* 2013

Proposed source(s) of the agent

The accession of *B. eupatorii* used in all host-specificity tests performed in the QC3 Microbiological area of the CSIRO Black Mountain Containment Facility in Canberra (QAP A1280) (Import permit no. IP11016131) originated from Los Nogales (Lat. 19.860217, Long. -102.156233), on the road between Zamora and Morelia, in the Michoacan Province of Mexico. This accession was found to infect all Australian crofton weed accessions (ex. Blue Mountains, Wollongong, Lord Howe Island) available for testing.

Agent's potential for control of the target

Baeodromus eupatorii infects young leaves and stems of crofton weed. It obtains nutrients and water from the host plant by establishing an intimate contact with living cells. Through this continuous absorption or diversion of assimilates from the host plant, the fungus becomes detrimental to plant development and reproduction (Fig. 2). The fungus also destroys leaf tissue by producing fruiting bodies thus reducing the photosynthetic surface of the plant.

Other rust fungi of weeds, such as *Puccinia chondrillina* on skeleton weed (Cullen 2012), *Puccinia myrsiphylli* on bridal creeper (Morin and Scott 2012) and *Maravalia cryptostegiae* on rubber vine (Palmer and Vogler 2012) have proved to be very effective biological control agents in Australia.

Information on non-target organisms at risk from the agent

Baeodromus eupatorii has only been reported from *Ageratina* and *Eupatorium* species (Farr et al. 2013). It is not known from other Central American species from the Asteraceae or any other plant family.

In Australia, there are no crop plants in the Asteraceae tribe Eupatorieae, to which crofton weed belongs (Appendix 1), although there is growing interest in the commercial potential of *Stevia rebaudiana* to produce natural sweetening agents. There are many species within the tribe that are reported as weeds in Australia. Other species have been introduced to Australia for horticultural purposes and many of them are reported as weeds in other countries (Randall 2007). There are only two species indigenous to Australia within the tribe: *Adenostemma lavenia* and *Adenostemma macrophyllum* (Orchard 2011).

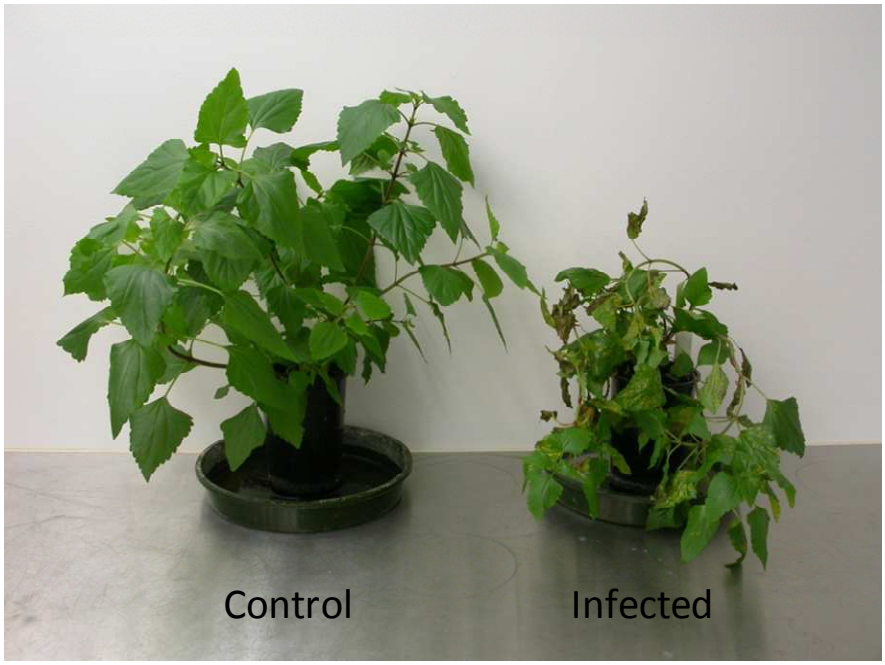

Figure 2 Example of impact of *Baeodromus eupatorii* on crofton weed (*Ageratina adenophora*). Infected plant on the right was artificially inoculated with the rust once a week for 5 consecutive weeks under controlled environment conditions in the quarantine facility. Photo taken at 4 weeks after the last inoculation.

Information and results on any other similar assessments undertaken on the species

Baeodromus eupatorii has not been used as a biological control agent anywhere. A culture of *B. eupatorii* (ex Mexico) was established in 2009 by South African colleagues at the Plant Protection Research Institute in Stellenbosch, but the fungus was not investigated further because the culture could not be maintained Heystek et al. 2011). No other risk assessment on *B. eupatorii*, other than that presented in this release application, has been undertaken before.

Possible interactions, including conflict-of-interest with existing biological control programs

Cruttwell McFadyen (2012) provides a review of previous biological control agents introduced for crofton weed in Australia. The gall-forming fly *Procecidochares utilis* was released in 1952 for the biological control of crofton weed (Dodd 1961). It produces swellings (galls) on stems which eventually kill them. Parasitism

of *P. utilis* larvae by a native parasitoid has been reported. Level of galling is usually too low for this agent to have a substantial negative impact on the weed (Auld 1969). A native crown-boring insect (*Dihammus argentatus*) is also reported to attack crofton weed in Australia and to kill plants in some situations (Auld 1969).

The fungus *Passalora ageratinae* (previously referred to as *Cercospora eupatorii, Phaeoramularia eupatorii-odorati* or *Mycovellosiella eupatorii*) (Crous et al. 2009) was accidentally introduced with the gall-fly in the 1950s (Cruttwell McFadyen 2012). It causes large necrotic leaf spots on older leaves of crofton weed, which coalesce and lead to leaf abscission (Auld 1969). It has been reported to kill seedlings during favourable seasons in Queensland (Haseler 1965), which may have helped decrease the rate of spread of the weed (Auld 1969).

The combined effect of these organisms is believed to have reduced the overall vigour of crofton weed and density of populations across its range in Australia (Cruttwell McFadyen 2012), although it remains a problem in some areas. Direct competition between *B. eupatorii* and the other natural enemies of crofton weed are unlikely to occur if the rust is released in Australia since they occupy different niches on plants. Indeed, we expect an additive or even synergistic interaction between these organisms, especially the two pathogens. Young leaves will first be infected by *B. eupatorii* and subsequently colonised by *P. ageratinae* as they age, thus causing more rapid defoliation.

Information on where, when and how initial releases will be made

Upon obtaining approval to release *B. eupatorii* in Australia, large numbers of crofton weed plants, maintained in the CSIRO glasshouses at Black Mountain, Canberra will be inoculated with plant material bearing telia removed from the quarantine facility. Infected crofton weed plants in the glasshouse will then be used to establish infections in the field at selected sites across the weed's range on mainland NSW and Queensland. Disease development and spread will be closely monitored during the first growing season. All available information on the rust, including host-specificity testing data and initial damage data collected on the mainland will be presented to the Lord Howe Island Board prior to introduction of *B. eupatorii* on the island.

Redistribution of *B. eupatorii* from infected to non-infected sites may be necessary since rust's basidiospores are fragile and not known to travel long distances on wind currents.

Information on target species in Australia

Taxonomy

Subclass:	Asteridae
Order:	Asterales
Family:	Asteraceae
Subfamily:	Asteroideae
Tribe:	Eupatorieae

Genus:	*Ageratina*
Species:	*adenophora* (Spreng.) R. M. King & H. Rob.
Common name:	Crofton weed
Synonyms:	*Eupatorium adenophorum*, *Ageratina trapezoides*, *Eupatorium trapezoideum*, *Eupatorium glandulosum, Eupatorium cognatum*

Description

Crofton weed is an erect and multi-stemmed perennial herb that grows 1–2 m high. It has purplish, smooth stems and broadly trowel-shaped (5-8 cm long and 3-7.5 cm wide), opposite, 3-nerved dark green leaves that are toothed along the apical margins (Parsons and Cuthbertson 2001, Muniappan et al. 2009). It produces small white florets that are clustered in groups of 50 to 70, forming 5–6 mm diameter heads. It reproduces by seed or vegetatively from its rootstock. Each plant can produce between 10 000 and 100 000 seeds per year. Seeds are windborne over long distances, which allow the weed to invade previously non-infested areas. Rooting from bent over or broken stems is frequently observed and contributes to local densification and extension of infestations.

Native range

Crofton weed is native of Mexico (Henderson 2006) and more specifically southern Mexico according to the Germplasm Resources Information Network (GRIN) database (http://www.ars-grin.gov/cgi-bin/npgs).

Distribution

Crofton weed was introduced from England to Sydney, NSW, as an ornamental around 1875 and was first collected as a garden escape on Sydney's North Shore in 1904 (Parsons and Cuthbertson 2001). From there it colonised the NSW North Coast and the south-east corner of Queensland in the early 1920s. Populations of the weed exploded in the 1940s and 1950s, when it began invading large areas of dairy pastures and horticultural land (Parsons and Cuthbertson 2001). The current distribution of crofton weed is presented in Figure 3.

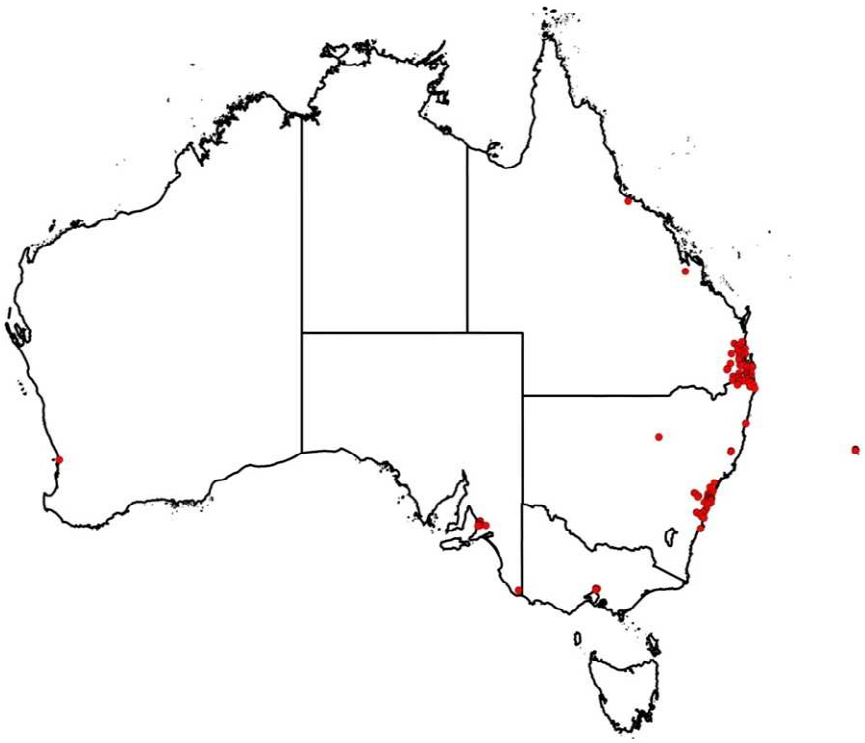

Figure 3 Current distribution of crofton weed (*Ageratina adenophora*) (Reproduced from Australia's Virtual Herbarium with permission of the Council of Heads of Australasian Herbaria Inc; map generated on 4 March 2013).

Summary of economic and environmental losses caused by the target

Crofton weed is a rapid-spreading weed that is invasive in many areas along the eastern coast of Australia, particularly cleared land that is not grazed, such as public reserves. Its ability to colonise steep sloping lands via windborne seeds preclude the use of mechanical or high volume herbicide treatments (Parsons and Cuthbertson 2001). It is reported to reduce crop yield and carrying capacity of grazing land and restrict movement of stock and machinery (Parsons and Cuthbertson 2001), but no assessment of the cost to agriculture in Australia has been conducted. It is unpalatable to cattle, but can be eaten by goats and sheep without apparent ill effects providing other pasture species are present. It is poisonous to horses, causing the 'Numinbah' or 'Tallebudgera' disease, which can take many years to become evident but is generally fatal (O'Sullivan 1979).

Crofton weed is reported to negatively impact on native flora, possibly through the release of allelopathic compounds (Zheng and Feng 2005, Zhu et al. 2011). For example, it is recognised as one of the threats to the endangered *Brachyscome ascendens*, currently known from only one location in NSW, on the Tweed Escarpment in the Border Ranges National Park (http://www.environment.nsw.gov.au/determinations/BrachyscomeAscendensAPerennialDaisyEndSpListing.htm).

Crofton weed is widespread and at high density in many areas of Lord Howe Island (LHI), an island off the NSW coast, declared a World Heritage Area in 1982 in recognition of its outstanding natural beauty and exceptional biodiversity. It is one of the two dominant weeds on LHI that has not been included in the eradication program implemented by the LHI Board since 2004 (S. Bower, LHI Board, pers. comm.). Infestations of dense, tall crofton weed plants hamper effective control and removal of highly invasive

plants such as glory lily (*Gloriosa superba*) on the lower slopes of the southern mountains and cherry guava (*Psidium cattleianum* var. *cattleianum*) in low to high elevations of the island, by making access difficult and reducing visibility, thereby effectively harbouring the targets.

Crofton weed prefers moist sites, high nutrient basalt soils and poses a particularly severe threat to native flora in the southern mountains of LHI. Landslips are a significant feature of the island's landscape. Crofton weed, with its wind-dispersed seed, often takes advantage of these large-scale natural disturbances and readily colonises these areas, preventing native fern, herb and tree species regeneration and succession (Auld and Hutton 2004). Invasion by crofton weed poses a major threat to intact plant communities such as the Mixed Fern and Herbfield Community, which is one of the most significant vegetation communities on the island. A host of threatened and endemic species are also at risk from crofton weed invasion, including the critically endangered twiner *Calystegia affinis*. This endemic species is sporadically found between 300 to 600m ASL at the base of cliff lines, following rock falls that open up new niches with increased light conditions. Crofton weed rapidly colonises these gaps and readily outcompetes *C. affinis*.

Other control methods available

Options for control of crofton weed include mechanical or chemical methods (Parsons and Cuthbertson 2001). The weed can be controlled by slashing followed by ripping or ploughing and then sowing suitable grasses or legumes. Several herbicides are currently registered for control of crofton weed. They are most effective when applied during late summer and autumn. A combination of slashing and herbicide applications on the regrowth followed by sowing with competitive species is recommended to restore productivity of infested land (Trounce and Dyason 2003).

Biological control is believed to be the only viable option to reduce densities of crofton weed, particularly in areas difficult to access across its range on the mainland and Lord Howe Island. Indeed Auld and Hutton (2004) argued that research into the biological control of crofton weed should be a national priority given the number of Lord Howe Island endemic plant species it threatens.

Information on all other relevant Commonwealth, State and Territory legislative controls of the target species

Crofton weed is declared as a Class 4 noxious weed in 25 coastal Local Control Authorities in NSW under the *NSW Noxious Weeds Act 1993*, which requires landholders to comply to the following: 'The growth of the plant must be managed in a manner that reduces its numbers, spread and incidence and continuously inhibits its reproduction' (http://www.dpi.nsw.gov.au/agriculture/pests-weeds/weeds/noxweed). Although widespread in South East Queensland, crofton weed is not a declared species under Queensland legislation, but may be declared under local government law (http://www.daff.qld.gov.au/4790_7246.htm).

Whether and when the target species was approved as a target species, and the proposing organisation

Crofton weed was targeted for biological control prior to 1984 when the current endorsement process was introduced. It and other early target weeds are included with a list of weeds endorsed as targets for biological control (see www.weeds.org.au/target.htm).

Host-specificity testing of *Baeodromus eupatorii*

Test list

The list of non-target plant species (60) used to test the specificity of *B. eupatorii* was compiled according to the phylogenetic centrifugal approach of Wapshere (1974), which places greater representation on the more closely-related species to the target weed. 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).

The recent published molecular phylogeny of Asteraceae (Funk et al. 2009) was used to devise the test list so that species most closely related to crofton weed that are present in Australia were given priority. The test list comprised a large number of species within the Eupatorieae, the tribe that crofton weed belongs to (Table 2). The list also included representative species in other tribes related to Eupatorieae within the sub-family Asteroideae that are present in Australia.

Materials and methods

TEST PLANTS

Crofton weed plants (Woollongong–Windy Gully accession; -34.26116, 150.47503) were propagated from stem cuttings treated with a hormone rooting gel (4 g l^{-1} indole-butyric acid), planted in a 1:1 perlite and vermiculite mixture, placed in the glasshouse and maintained wet with intermittent overhead misting to encourage root development prior to planting into potting mixture (5:1:1:3 straw-based compost, peat moss, river sand, perlite, with 1.4 kg slow-release fertilizer m^{-3} [Aboska®, N:P:K 15.16:6.93:5.19]). Plants were maintained in a glasshouse (16–26°C; natural light and, if required, additional lighting with metal halide lights to maintain a 12-h photoperiod) and fertilised fortnightly with liquid fertiliser (Aquasol™; N=23, P=4, K=18).

The various non-target plant species to be tested (Table 2) were propagated from seeds (obtained from commercial outlets or from the field) or cuttings from field plants, or obtained as whole plants from the field or nurseries, and grown in the glasshouse (conditions as above). Actively-growing plants were taken into the QC3 area of the Black Mountain Containment Facility for testing.

PRODUCTION OF RUST INOCULUM

Every week, two to four large crofton weed plants (up to 50 cm in height including pots – 10-15 cm diam.) were inoculated with *B. eupatorii* to maintain a continuous supply of inoculum for host-specificity tests. Leaf discs (4 mm diam) with one sorus of mature telia were cut from infected crofton weed plants (approx. 4 wks after inoculation) and each deposited onto the slightly melted surface of a 2% water agar block (approx. 7 mm^2) placed in the base of a 9 mm diam Petri dish (telia uppermost; two blocks per dish placed at opposite side). The dishes containing telia (without lids) were then fixed with sticky tape to the inside bottom of 25 L opaque plastic buckets (four dishes per bucket). Each bucket with telia was inverted over the opening of another 25 L bucket containing one or two crofton weed plants that had been misted with distilled water. The inverted bucket was secured to the other bucket with sticky tape and placed in a controlled-environment room at 20°C for 48 h. During that 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 controlled-environment room (12 h photoperiod, fluorescent

lights). At 14 days after the beginning of the inoculation period, a fine camel hair brush was used to cross-fertilise pycnia by transferring mucus containing pycniospores between them. This manual cross-fertilisation ensured that a large number of telia developed on the under surface of leaves.

Table 2 List of non-target species present in Australia that were used to test the specificity of *Baeodromus eupatorii*.

TRIBE		SPECIES	COMMON NAME [1]	STATUS IN AUSTRALIA [2]
Eupatorieae	1	*Adenostemma lavenia*	Sticky daisy	Native
	2	*Adenostemma macrophyllum*		Native
	3	*Ageratina adenophora*	Crofton weed	Introduced and biocontrol target
	4	*Ageratina altissima*		Introduced and horticultural
	5	*Ageratina ligustrina*		Introduced and naturalised
	6	*Ageratina riparia*	Mistflower, William Taylor	Introduced and weed
	7	*Ageratum conyzoides*	Billygoat plant	Introduced and weed
	8	*Ageratum houstonianum*	Blue billygoat weed	Introduced and weed
	9	*Bartlettina sordid*	Blue mist plant, Purple torch	Introduced and garden escape
	10	*Chromolaena odorata*	Siam weed	Introduced and weed
	11	*Conoclinium coelestinum*	Blue mistflower	Introduced and horticultural
	12	*Eupatorium cannabinum*	Hemp agrimony	Introduced
	13	*Eutrochium purpureum* var. *purpureum*	Joe-pye weed	Introduced and herbal
	14	*Gymnocoronis spilanthoides*	Senegal tea	Introduced and weed
	15	*Liatris spicata*	Gayfeather	Introduced and horticultural
	16	*Mikania micrantha*	Mile-a-minute	Introduced and weed
	17	*Praxelis clematidea*	Praxelis	Introduced and weed
	18	*Stevia ovata*	Candyleaf	Introduced
	19	*Stevia rebaudiana*	Stevia, Sweet leaf	Introduced and horticultural
Tribes that are closest to Eupatoriae in the Heliantheae alliance [3]				
Heliantheae	20	*Ambrosia artemisiifolia*	Ragweed	Introduced and weed
	21	*Eclipta prostrate*	White eclipta	Native
	22	*Helianthus annuus* Sunbird 7	Sunflower	Introduced and crop
	23	*Helianthus annuus* Hyoleic 41	Sunflower	Introduced and crop
	24	*Melanthera biflora* (=*Wollastonia biflora*) [4]		Native
	25	*Parthenium hysterophorus*	Parthenium weed	Introduced and weed
	26	*Xanthium occidentale*	Noogoora burr	Introduced and weed
	27	*Zinnia elegans* (Early wonder mixed)	Zinnia	Introduced and horticultural
Madieae	28	*Arnica Montana*		Introduced and herbal
Millerieae	29	*Guizotia abyssinica*	Niger seed	Introduced and naturalised
	30	*Sigesbeckia orientalis*	Cobber weed	Native [5]
	31	*Tridax procumbens*	Tridax daisy	Introduced and weed
Other tribes in the Heliantheae alliance [5]				
Bahieae	32	*Schkuhria pinnata*	Dwarf marigold	Introduced and naturalised [6]
Coreopsideae	33	*Bidens pilosa* (=*Coreopsis leucantha*)	Cobbler's-pegs	Introduced and weed
	34	*Cosmos bipinnatus* (=*Bidens formosa*) (Cosmos Purity)	Cosmos	Introduced and weed

	35	*Dahlia variabilis* (Cactus Flowered Mix)	Dahlia	Introduced and horticultural
	36	*Glossocardia bidens* (=*Glossogyne tannensis*)	Cobbler's tack	Native
Helenieae [6]	37	*Gaillardia aristata* x *Gaillardia pulchella* (=*Gaillardia* x *grandiflora*) (Choice Mix)	Gaillardia	Introduced and naturalised
	38	*Gaillardia pulchella* (Sundance Mix)	Gaillardia	Introduced and naturalised
Neurolaeneae	39	*Enydra fluctuans*		Native
Tageteae	40	*Flaveria australasica*	Yellow daisy	Native
	41	*Tagetes erecta*	Marigold	Introduced and horticultural
	42	*Tagetes patula* (Petite Yellow)	Marigold	Introduced and weed
Closest tribes to the Heliantheae alliance				
Athroismeae	43	*Centipeda minima*	Spreading sneezeweed	Native
Inuleae	44	*Pluchea* sp.		Native
Other tribes in the Asteroideae sub-family				
Astereae	45	*Brachyscome segmentosa* [4]	Lord Howe Island daisy	Native
	46	*Olearia ballii* [4]	Mountain daisy	Native
	47	*Olearia mooneyi* [4]	Pumpkin bush	Native
	48	*Olearia elliptica* [4]	Sticky daisy-bush	Native
Anthemideae	49	*Chrysanthemum* × *morifolium* (Autumn glory mix)	Chrysanthemum	Introduced and horticultural
	50	*Glebionis coronarium* (=*Chrysanthemum coronarium*) (Double flowered Mix)	Chrysanthemum	Introduced and weed
	51	*Tanacetum vulgare*	Tansy	Introduced and weed
Calenduleae	52	*Calendula officinalis* (Greenheart orange)	Garden marigold	Introduced and weed
	53	*Chrysanthemoides monilifera* spp. *rotundata*	Bitou bush	Introduced and weed
	54	*Dimorphotheca sinuate*	African daisy	Introduced and weed
Gnaphalieae	55	*Cassinia tenuifolia* [4]	Bully bush	Native
	56	*Vellereophyton dealbatum* (=*Gnaphalium candidissimum*)	Cubweed	Introduced and weed
	57	*Xerochrysum bracteatum* (=*Helichrysum bracteatum*) (Tall mix)	Golden everlasting, strawflower	Native
Senecioneae	58	*Lordhowea insularis* [4]		Native
	59	*Senecio howeanus* [4]		Native
	60	*Senecio pauciradiatus* [4]		Native
	61	*Senecio pinnatifolius* var. *lanceolatus* (=*Senecio lautus*)	Lanceleaf coast groundsel	Native

Source: Tribe classification based on Funk et al. (2009).

[1] Common names obtained from Australian Plant Name Index (http://www.anbg.gov.au/apni/) or NSW Flora Online (http://plantnet.rbgsyd.nsw.gov.au), where available.
[2] Weed status according to Randall (2007).
[3] Tribes Perityleae (only six genera) and Polymnieae (only one genus) do not contain any Australian native species (Flora of Australia Online – Asteraceae; http://www.anbg.gov.au/abrs/online-resources/flora). According to Randall (2007) only *Perityle emoryi* (Perityleae) and *Polymnia connata* (Polymnieae) have been introduced to Australia.
[4] Endemic on Lord Howe Island.
[5] Status according to NSW Flora Online.
[6] Tribes Chaenactideae (only three genera) and Feddeeae (only one genus) do not contain any Australian native species (Flora of Australia Online – Asteraceae). According to Randall (2007) only *Chaenactis nevadensis* (Chaenactideae) has been introduced to Australia.

HOST-SPECIFICITY TESTS

Each plant species was tested in two separate trials to account for any possible variation in time, except for *Liatris spicata* and *Olearia mooneyi*, which were only tested once because of lack of material (Table 2). Plants (up to 30 cm in height including pot) were chosen for each trial based on the presence of new, young growth (five plant replicates per species per trial unless indicated otherwise). Thirty-two trials consisting of up to eight species each, including the positive control crofton weed, were performed. Five agar blocks with leaf discs bearing mature telia of *B. eupatorii* (as described above in section 'Production of rust inoculum') were placed in the base of a 9 mm diam Petri dish. The dish with telia (without a lid) was fixed with sticky tape to the inside bottom of a 10 L opaque plastic bucket, which was then inverted over the opening of another 10 L bucket that contained one plant misted with distilled water and placed in a controlled-environment room (conditions as above). To verify that the telia used for inoculation produced basidiospores, on extra dish with telia on agar blocks was covered with its lid, sealed with parafilm, inverted on the bench of the controlled-environment room and covered with an empty bucket. After 48 h, the inside of the lid of the extra dish with telia was examined under a stereomicroscope for presence of basidiospores and plants were removed from the buckets and placed on the bench of the controlled-environment room. Plants were examined 14 days after the beginning of the inoculation period for presence of macroscopic disease symptoms and assessed again at 28 days to allow for any possible delayed symptoms to develop. For species that developed pycnia, manual cross-fertilisation of pycnia was performed (as described above) at 14–21 days after the beginning of the inoculation period to determine if telia developed.

MICROSCOPIC EXAMINATIONS

Additional inoculations targeted at single leaves of test plants were performed in parallel with the host-specificity trials to provide material for microscopic examination of rust development. Leaf discs (7 mm diam) with one or more sori of mature telia of *B. eupatorii* were cut from infected crofton weed plants (approx. 4 weeks after inoculation), then cut in half and each piece placed on an agar block (as described above in section 'Production of rust inoculum'). Four blocks with telia were placed in a row in the middle of the base of 5 mm diam Petri dishes (telia uppermost). 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) each on one plant of each test species (Fig. 4). 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 controlled-environment 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 controlled-environment room.

Figure 4 Experimental set up for inoculation of single leaves with telia of *Baeodromus eupatorii* to provide material for microscopic examination of rust development.

For each plant species, one of the inoculated leaves or groups of small leaves was excised 4–6 days after the beginning of the inoculation period and cut into small pieces (0.5–1 cm^2). The pieces were cleared and stained in a solution containing aniline blue, ethanol, chloroform, lactic acid, phenol and chloral hydrate for 48 h (Bruzzese and Hasan 1983). They were then rinsed in water, placed in a saturated solution of chloral hydrate for 1 day 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. At least 50 basidiospores per species were examined. The other inoculated leaf or group of small leaves were left on the plant and examined for macroscopic symptoms at 14 days after the beginning of the inoculation period.

ASSESSMENT OF RUST DEVELOPMENT

The microscospic development of *B. eupatorii* and macroscopic symptoms on test plants were assessed according to 19 categories (Fig. 5). The susceptibility of the test plant species to the rust was then classified according to seven categories based on systems devised by Mortensen (1985), Bruzzese and Hasan (1986) and Evans and Tomley (1994) (Table 3). The susceptibility rating of each species is based on the most advanced developmental stage of the rust observed.

COMPLETION OF LIFE CYCLE ON SUSCEPTIBLE HOSTS

An additional experiment using a very high inoculum load was conducted with species that developed macroscopic disease symptoms in previous tests. Strips (approx. 1 cm wide) were cut from three large infected crofton weed leaves with a high density of sori of mature telia and fixed with sticky tape to the inside bottom of a 25 L bucket. The inside of the bucket with telia was misted with distilled water and inverted over the opening of another 25 L bucket containing two plants of the test species and one crofton weed plant that was misted with distilled water. The inverted bucket was secured to the other bucket with sticky tape and placed in a controlled-environment room (conditions as above) for 48 h. Plants were then removed from the bucket and placed on the bench of the room.

At 9 days after the beginning of the inoculation period, young (2^{nd} or 3^{rd} from growing point) and older (6^{th} to 8^{th} from growing point) leaves from each of the plant species were excised and samples for microscopic examination taken and processed as above.

At 14 days after the beginning of the inoculation period, pycnia (if present) on each plant were cross-fertilised as described above. At 21 days after the beginning of the inoculation period, infected leaves with many sori of mature telia were excised from the test species and crofton weed plants. One infected leaf from each species was deposited (telia uppermost) onto the slightly melted surface of 2% water agar contained in a 9 mm diam Petri dish (one leaf per dish). Each plate was then inverted over the lid of the dish into which a microscope glass slide with a large water agar block (2×4 cm) had been placed. The dishes were sealed with parafilm, wrapped in aluminium foil and placed in the controlled-environment room (conditions as above). After 48 h, blue-lacto-glycerol stain was applied to the agar blocks prior to examination with a light microscope to determine if basidiospores were present and had germinated.

Additional infected leaves (14–20) from the test species and crofton weed were fixed with sticky tape to the inside bottom of two different 25 L buckets. The inside of each bucket with telia from a different host species was misted with distilled water and inverted over the opening of another 25 L bucket containing one plant of the test species and one crofton weed plant that was misted with distilled water. Buckets were secured in place with sticky tape and incubated as above. Plants were removed from the buckets after 48 h, placed on the bench of the controlled-environment room and examined for presence of pycnia 14 days after the beginning of the inoculation period.

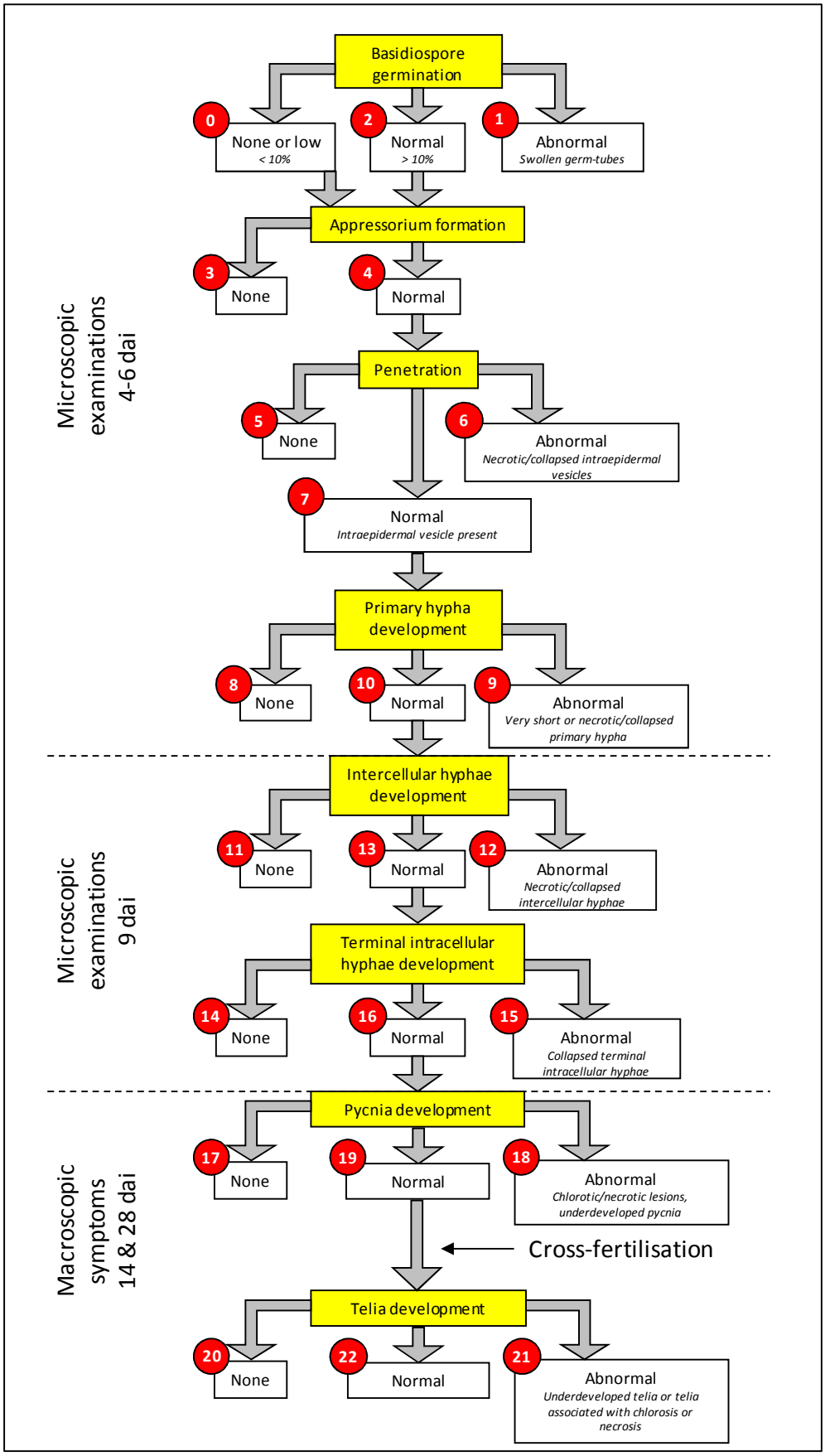

Figure 5 Schematic representation of the categories used to assess the microscopic development of *Baeodromus eupatorii* and macroscopic symptoms on the test plant species (dai = days after the beginning of the inoculation period).

Table 3 Categories used to classify the susceptibility of test plant species to *Baeodromus eupatorii*.

CATEGORIES	MACROSYMPTOMS	DEVELOPMENTAL STAGE OF THE RUST AND MICROSYMPTOMS
Immune (I)	None	No sign of penetration
Highly resistant (HR)	None	Abnormal penetration (intraepidermal vesicle necrotic/collapsed or normal, but primary hypha either very short, necrotic/collapsed or absent); plant defence reactions sometime visible at the cellular level.
Resistant (R)	Discolouration, chlorosis sometime present.	Successful penetration and development of normal primary hypha and some intercellular hyphae. No terminal intracellular hyphae present.
Moderately resistant (MR)	Chlorotic or necrotic spots present.	Restricted network of intercellular hyphae developed. Terminal intracellular hyphae present, but generally collapsed. Plant host cell plasmolysis often present.
Moderately susceptible (MS)	Chlorotic or necrotic spots present. Underdeveloped pycnia present. No mucus with pycniospores present.	Extensive network of intercellular hyphae; terminal intracellular hyphae abundant but often collapsed. Development of pycnia initiated but aborted or incomplete.
Susceptible (S)	Normal pycnia present but restricted in numbers. Mucus with pycniospores present. Telia sometimes underdeveloped and often associated with chlorosis or necrosis following cross-fertilisation of pycnia.	Extensive network of intercellular hyphae; abundant well-developed terminal intracellular hyphae.
Highly susceptible (HS)	Large numbers of normal pycnia present on most of the young foliage. Mucus with pycniospores present. Normal telia developed following cross-fertilisation of pycnia.	Extensive network of intercellular hyphae; abundant well-developed terminal intracellular hyphae.

Results

The full range of developmental stages of *B. eupatorii* observed on crofton weed and on each of the test plant species is presented in Table 4. In all host-specificity tests, basidiospores were produced from telia used for inoculation and all control crofton weed plants developed pycnia.

RUST DEVELOPMENT ON SPECIES WITH MACROSCOPIC SYMPTOMS

Only crofton weed and two other *Ageratina* species (*A. altissima* and *A. riparia*) developed macroscopic disease symptoms across the trials. Crofton weed and *A. altissima* were rated as highly susceptible to *B. eupatorii* on the basis of the most advanced developmental stage of the rust observed across all trials (Table 4). These two species developed abundant pycnia on most young leaves and stems of inoculated plants (Figs 6E, 7E,F). Following cross-fertilisation, these pycnia developed normal telia on the under surface of leaves. When exposed to high humidity, these telia produced abundant basidiospores that germinated in vitro and also infected their host plant, leading to the production of pycnia. This confirmed that *B. eupatorii* can complete its life cycle on these two *Ageratina* species.

In contrast, *A. riparia* developed pycnia infrequently and in low numbers on young leaves and was consequently rated as susceptible to *B. eupatorii* (Table 4). The telia produced after cross-fertilisation of

Table 4 Microscopic development of *Baeodromus eupatorii* and macroscopic symptoms on each of the test plant species inoculated with the fungus, based on categories described in Figure 5. Susceptibility ratings were evaluated for each test plant species according to the categories presented in Table 3.

SPECIES [1]	MICRO/MACROSYMPTOMS																							RATING
	GERMINATION					PENETRATION			COLONISATION					REPRODUCTION										
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
Adenostemma lavenia	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Adenostemma macrophyllum	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Ageratina adenophora	–	–	+	–	+	–	–	+	–	–	+	–	–	+	–	–	+	–	–	+	–	–	+	HS
Ageratina altissima [2]	–	–	+	–	+	–	–	+	–	–	+	–	–	+	–	–	+	–	–	+	–	–	+	HS
Ageratina ligustrina [3]	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Ageratina riparia	–	–	+	–	+	–	–	+	–	–	+	–	–	+	–	–	+	–	–	+	–	–	+	S
Ageratum conyzoides	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Ageratum houstonianum	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Ambrosia artemisiifolia	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Arnica montana	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Bartlettina sordida	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Bidens pilosa	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Brachyscome segmentosa	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Calendula officinalis	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Cassinia tenuifolia [3]	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Centipeda minima	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Chromolaena odorata	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Chrysanthemoides monilifera spp. *rotundata*	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Chrysanthemum × morifolium	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Conoclinium coelestinum	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Cosmos bipinnatus	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Dahlia variabilis	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I

SPECIES [1]	MICRO/MACROSYMPTOMS																							RATING
	GERMINATION					PENETRATION			COLONISATION					REPRODUCTION										
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
Dimorphotheca sinuata	–	–	+	–	+	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	HR
Eclipta prostrata	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Enydra fluctuans	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Eupatorium cannabinum	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Eutrochium purpureum var. *purpureum*	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Flaveria australasica [2, 4]	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Gaillardia aristata x *Gaillardia pulchella*	–	–	+	–	+	–	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	HR
Gaillardia pulchella	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Glebionis coronarium	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Glossocardia bidens	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Guizotia abyssinica	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Gymnocoronis spilanthoides	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Helianthus annuus Hyoleic 41	No microscopic assessment performed; no macroscopic symptoms observed on inoculated plants																							I / HR
Helianthus annuus Sunbird 7	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Liatris spicata	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Lordhowea insularis [5]	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Melanthera biflora	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Mikania micrantha	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Olearia ballii	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Olearia mooneyi [6]	No microscopic assessment performed; no macroscopic symptoms observed on the sole plant available and inoculated																							I / HR
Olearia elliptica	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Parthenium hysterophorus	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Pluchea sp. [2]	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Praxelis clematidea	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I

SPECIES [1]	MICRO/MACROSYMPTOMS																							RATING
	GERMINATION					PENETRATION			COLONISATION						REPRODUCTION									
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
Schkuhria pinnata	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Senecio howeanus	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Senecio pauciradiatus	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Senecio pinnatifolius var. *lanceolatus*	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Sigesbeckia orientalis	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Stevia ovata [2]	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Stevia rebaudiana	–	–	+	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	HR
Tagetes erecta	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Tagetes patula	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Tanacetum vulgare	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Tridax procumbens	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Vellereophyton dealbatum	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Xanthium occidentale	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Xerochrysum bracteatum	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I
Zinnia elegans	–	–	+	–	+	–	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	HR

[1] All species were tested in two separate trials (five plant replicates per species per trial unless indicated otherwise), except *L. spicata* and *O. mooneyi* that were tested in a single trial due to lack of available material.
[2] Three replicates were used in one of the trials for this species.
[3] Four replicates were used in both trials for this species.
[4] Four replicates were used in one of the trials for this species.
[5] Three and two replicates were used in each of the trials for this species, respectively.
[6] One replicate was used in the trial for this species due to lack of material.

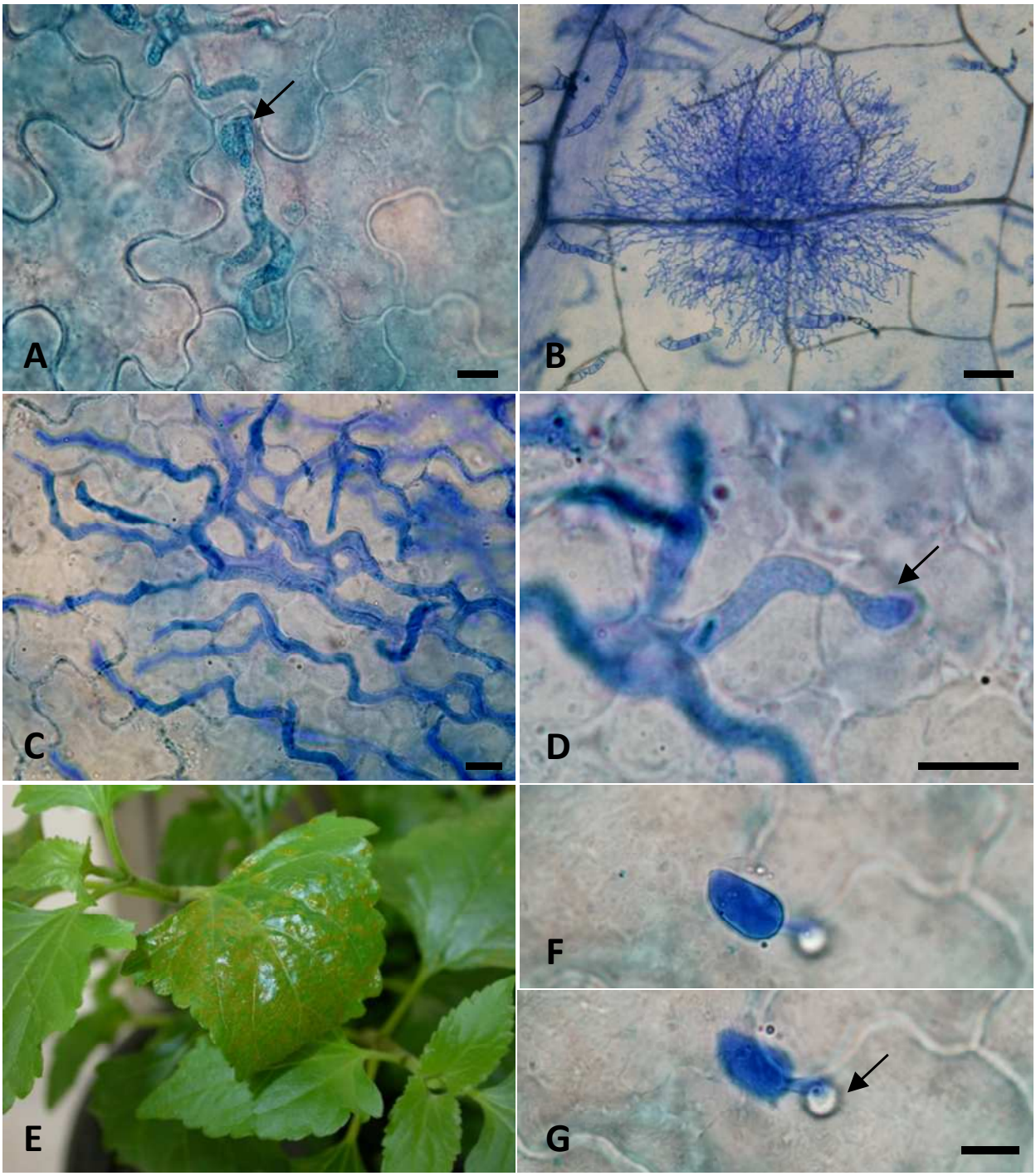

Figure 6 Development of *Baeodromus eupatorii* on young (A-E) and older (F-G) leaves of crofton weed (*Ageratina adenophora*) (bar B = 100 µm; all other bars = 10 µm; dai = days after the beginning of the inoculation period). A. Primary hypha in an epidermal cell at 4 dai. Arrow indicates point of penetration between epidermal cells. B. Infection site at 9 dai. C. Intercellular hyphae at the margin of an infection site at 9dai. D. An intercellular hypha from which a terminal intracellular hyphae (arrow) has developed in a host mesophyll cell at 9 dai. E. Pycnia with mucus containing pycniospores on upper leaf surface at 14 dai. F & G. Micrographs of a germinated basidiospore on an older leaf taken at different depths of field at 9 dai. A plant defence reaction (arrow) has prevented penetration of the leaf.

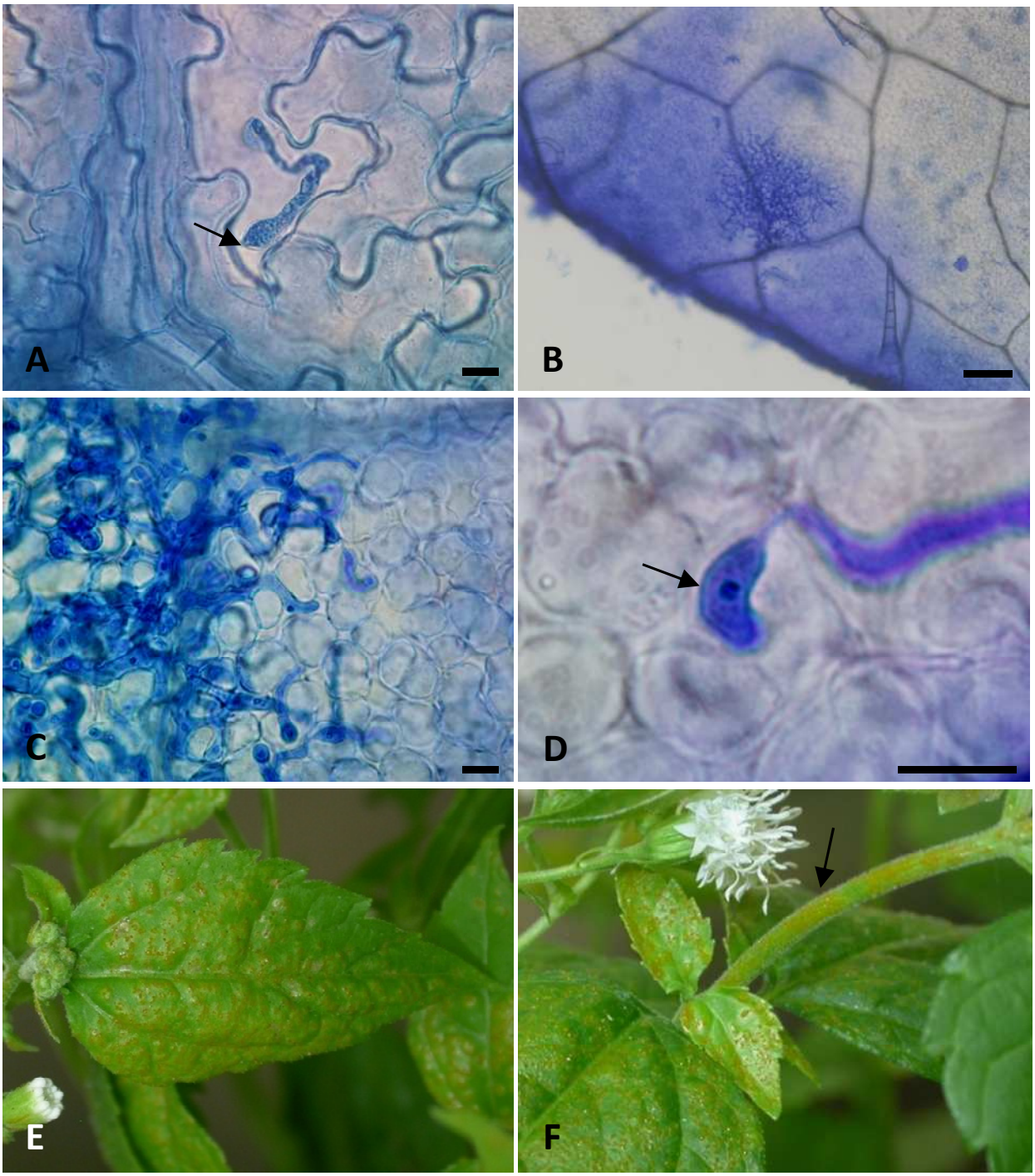

Figure 7 Development of *Baeodromus eupatorii* on young leaves of *Ageratina altissima* (bar B = 100 µm; all other bars = 10 µm; dai = days after the beginning of the inoculation period). A. Primary hypha in an epidermal cell at 4 dai. Arrow indicates point of penetration which did not occur between epidermal cells. B. Infection site at 9 dai. C. Intercellular hyphae at the margin of an infection site at 9dai. D. An intercellular hypha from which a terminal intracellular hyphae (arrow) has developed in a host mesophyll cell at 9 dai. E & F. Pycnia with mucus containing pycniospores on upper leaf surface at 14 dai. Note that pycnia also developed on the young stem (arrow) (F).

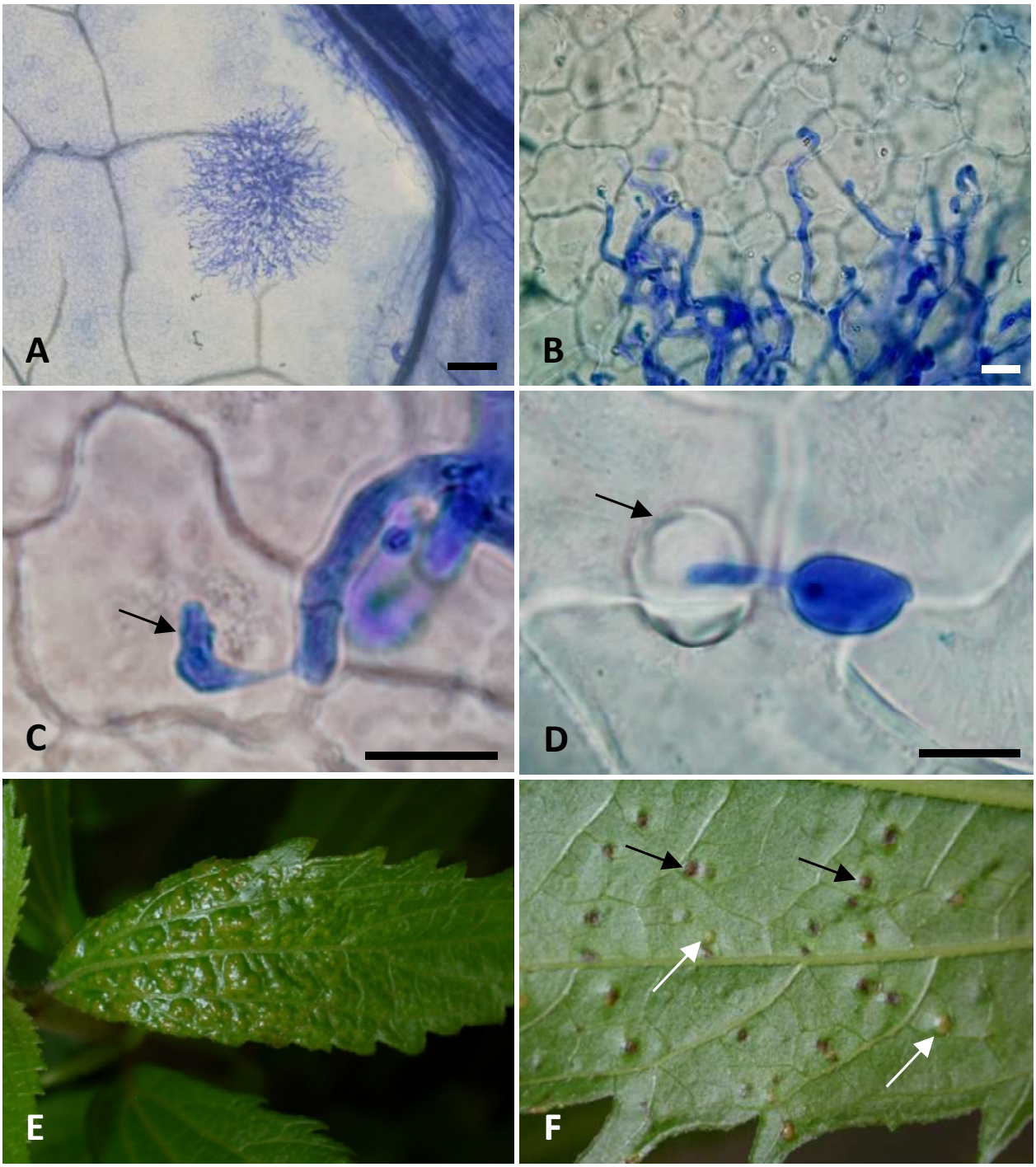

Figure 8 Development of *Baeodromus eupatorii* on young (A-C, E-F) and older (D) leaves of *Ageratina riparia* (bar A = 100 µm; all other bars = 10 µm; dai = days after the beginning of the inoculation period). A. Infection site at 9 dai. B. Intercellular hyphae at the margin of an infection site at 9dai. C. An intercellular hypha from which a terminal intracellular hyphae (arrow) has developed in a host mesophyll cell at 9 dai. D. A germinated basidiospore on an older leaf at 9 dai. A plant defence reaction (arrow) has prevented penetration of the leaf. E. Pycnia with mucus containing pycniospores on upper leaf surface at 14 dai. F. Normal telia (white arrows) observed on the under surface of an infected leaf at 28 dai. Some telia (black arrows) are underdeveloped and associated with necrosis.

these pycnia were often associated with necrosis (Fig. 8F). However, in the experiment in which a very high inoculum density was used, the rust did develop abundant pycnia on a couple of very young, still expanding leaves of this species (Fig. 8E). Following cross-fertilisation, these pycnia produced normal telia, which after exposure to high humidity produced basidiospores that germinated in vitro. Because of the limited amount of *A. riparia* material with normal telia it was not possible to carry out an experiment to confirm that the rust can complete its life cycle on this host.

Microscopic examinations of young leaves of crofton weed and *A. altissima* at 4 days after the beginning of the inoculation period revealed well-developed intraepidermal vesicles that had elongated into primary hyphae within epidermal cells as a result of successful penetration by *B. eupatorii* (Figs 6A, 7A). By 9 days after the beginning of the inoculation period, the rust had produced at infection sites an extensive network of intercellular hyphae (Figs 6B,C, 7B,C) with terminal intracellular hyphae (Figs 6D, 7D), from which the rust absorbs nutrients from its host.

Primary hyphae were not observed on any of the samples taken from *A. riparia* at 4 days after the beginning of the inoculation period. However, several well-developed infection sites with intercellular and terminal intracellular hyphae similar to those seen on crofton weed and *A. altissima* were observed on *A. riparia* inoculated with a very high inoculum density at 9 days after the beginning of the inoculation period (Fig. 8A-C).

No macrosymptoms developed on older leaves of crofton weed and the other two *Ageratina* species. Penetration by basidiospores that landed and germinated on older leaves of crofton weed and *A. riparia* was not successful and appeared to have been arrested by a plant defence reaction in the form of a callose around the penetration peg (Figs 6F,G, 8D).

RUST DEVELOPMENT ON OTHER SPECIES

No macroscopic symptoms, including chlorosis or necrosis, were observed on any of the other test species. *Baeodromus eupatorii* basidiospores germinated on the leaf surface of all these species (Table 4), producing germ tubes ranging from very short to elongated (Fig. 9A, B), and in one occasion, on *Tagetes patula*, germ tubes were slightly thicker than normal (Fig. 9C). The appressorium at the end of the germ tube consisted of a slight swelling at the point of contact with the leaf surface, which could only be detected on some germ tubes with light microscopy (Fig. 9A). On some species (e.g. *Brachyscome segmentosa*, *Dimorphotheca sinuata*), the peg from an appressorium that attempted penetration was prevented to develop any further by a plant defence reaction (Fig. 9D).

On highly resistant species (*D. sinuata*, *Gaillardia aristata x Gaillardia pulchella*, *Stevia rebaudiana*, *Zinnia elegans*; Table 4), collapsed intraepidermal vesicles that had not developed any further were observed by the time of the microscopic examination (Fig. 10A, B). Normal intraepidermal vesicles however, were also observed at penetration sites on *D. sinuata*, *G. aristata x G. pulchella* and *Z. elegans* (Fig. 10C, D). In some instances, primary hyphae begin to develop or had fully developed from these vesicles, but were collapsed by the time of the microscopic examination (Fig. 10E, F).

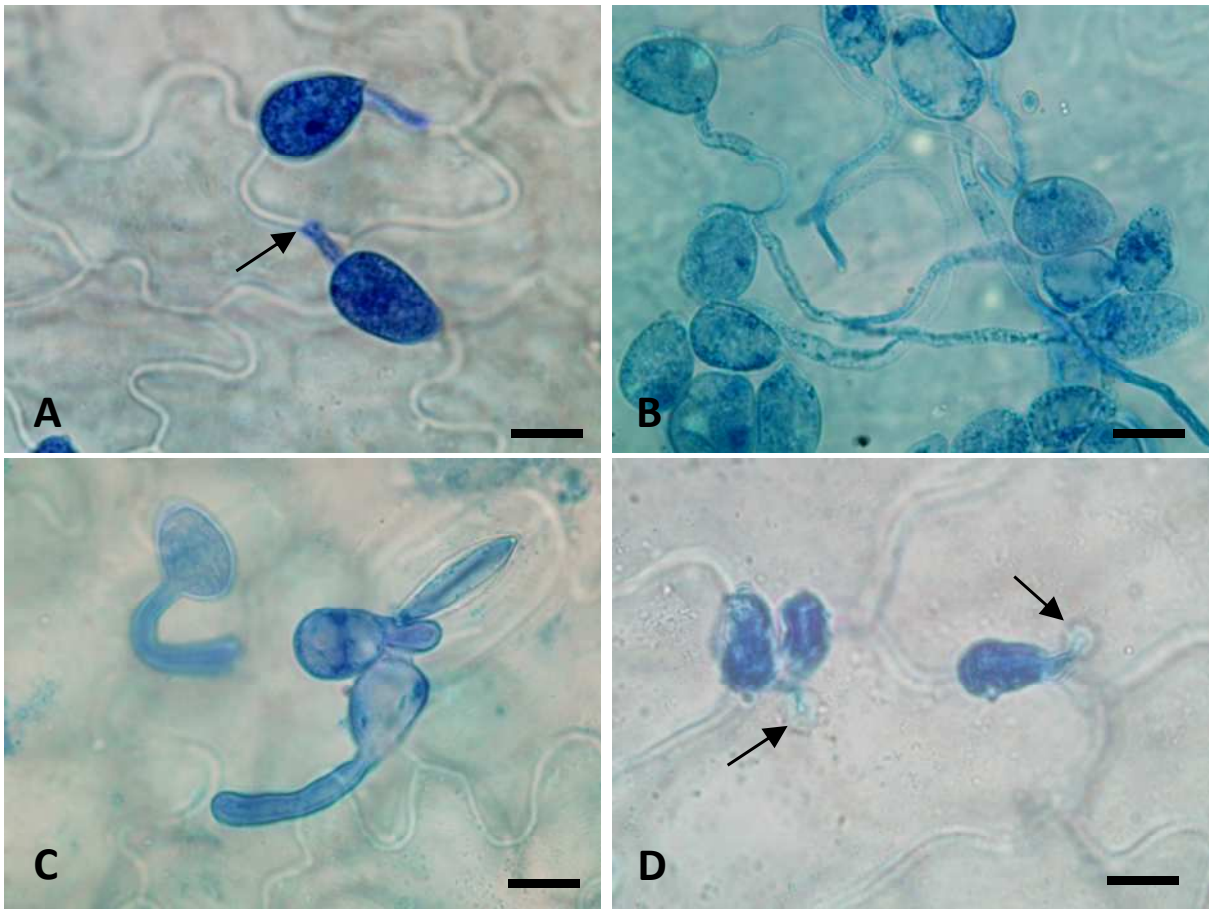

Figure 9 Germination of *Baeodromus eupatorii* basidiospores on young leaves of various plant species at 4 days after the beginning of the inoculation period (bars = 10 µm) A. Basidiospores with short, narrow germ-tubes on *Sigesbeckia orientalis*. Arrow indicates an appressorium, the slightly swollen end of a germ-tube at the point of contact with the leaf surface. B. Basidiospores with long germ-tubes on *Arnica montana*. C. Basidiospores with thick germ-tubes on *Tagetes patula*. D. Germinated basidiospores that have attempted penetration on *Dimorphotheca sinuata*. Penetration pegs were prevented to develop any further by a plant defence reaction (arrows).

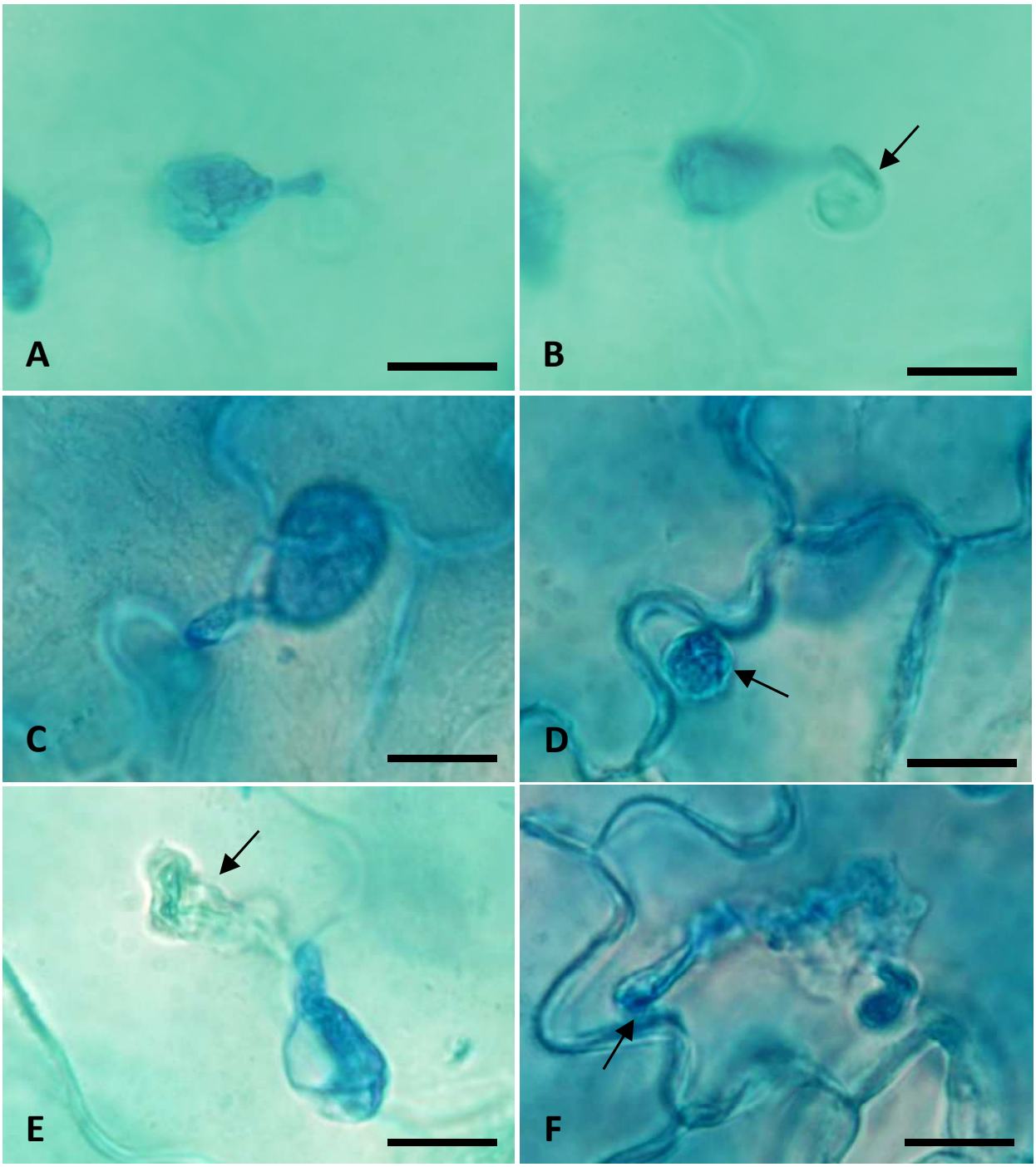

Figure 10 Development of *Baeodromus eupatorii* on young leaves of two non-host plant species at 4 days after the beginning of the inoculation period (bars = 10 µm) A & B. Micrographs of a germinated basidiospore on *Gaillardia aristata* x *Gaillardia pulchella* taken at different depths of field. The fungus has penetrated a plant cell but the intraepidermal vesicle has collapsed and no further development occurred. C & D. Micrographs of a germinated basidiospore on *Zinnia elegans* taken at different depths of field. The fungus has penetrated a plant cell and produced a normal intraepidermal vesicle (arrow). E. A germinated basidiospore on *G. aristata* x *G. pulchella*, which has successfully penetrated a plant cell but the intraepidermal vesicle and short primary hypha (arrow) have collapsed. F. A collapsed primary hypha (arrow) on *Z. elegans*.

Discussion

The results presented here demonstrate that *B. eupatorii* is a highly host specific rust fungus, being capable of successfully developing only on three species within the *Ageratina* genus (*A. adenophora*–crofton weed, *A. altissima*, *A. riparia*). Across all experiments, both crofton weed and *A. altissima* consistently supported development of abundant pycnia and telia, which produced basidiospores that were capable on infecting either species, demonstrating that the rust can complete its life cycle on these hosts. In contrast, *A. riparia* was not as suitable a host for *B. eupatorii* as these other two species. While the rust produced abundant pycnia on a few very young, still expanding leaves of this species when a very high density of inoculum was used, in the standard host-specificity trials pycnia were infrequently produced and telia were often associated with necrosis. It is therefore most unlikely that *A. riparia* alone could sustain a thriving population of the rust in the field. The lack of penetration and infection of *Ageratina ligustrina* was surprising since it belongs to the same genus of the susceptible hosts identified, further indicating the narrow host-range of this rust.

Based on the literature, *B. eupatorii* has only been found on *Ageratina* and *Eupatorium* species (Buriticá and Hennen 1980, Farr et al. 2013). It is noteworthy that there have been many problems with the generic delimitation of *Eupatorium* and recent molecular data (Schmidt and Schilling 2000) support the narrow delimitation of *Eupatorium* of King and Robinson (1987), which proposed that it be restricted to 42 species. Consequently, herbarium records of *B. eupatorii* collected in its native range of Central and South America that indicate as the host plant '*Eupatorium* sp.' without a species name are probably taxonomically inaccurate. Further, of the five *Eupatorium* species (including crofton weed) recorded as hosts of *B. eupatorii* in the literature (Buriticá and Hennen 1980, Farr et al. 2013), all, except *E. pycnocephalum* (syn. *Fleischumannia pycnocephala*), were reclassified as *Ageratina* species by King and Robinson (1987). On this basis and assuming that the *E. pycnocephalum* record may be a misidentification, it seems reasonable to propose that the host-range of *B. eupatorii* is most likely restricted to the *Ageratina* genus.

All other 58 non-target plant species tested, which included a large number of other representatives from the Eupatorieae tribe (including the two Australian native *Adenostemma* species) and representatives from across tribes related to Eupatorieae within the sub-family Asteroideae of the Asteraceae family that are present in Australia, were either rated as immune or highly resistant to the rust. Although conditions used during host-specificity testing were optimal for disease development on susceptible hosts, *B. eupatorii* did not succeed at infecting any of the other plant species tested. It is important to point out that such artificial conditions during host-specificity testing have been reported to predispose plants to infection by plant pathogens (e.g. Bruckart et al. 1985). The lack of penetration or arrested development of the rust following penetration or after initial infection structures were produced on these species were typical of reactions occurring as a result of non-specific, basic resistance mechanisms of non-host plants (Heath 1981).

Conclusion

The high specificity of *B. eupatorii* demonstrated in this study satisfies requirements from quarantine authorities for release into Australia. The possible infection of *A. riparia* in the field would not pose a problem since this species is an undesirable environmental weed in Australia, which is the target of a biological control program (Schooler et al. 2012). While the other susceptible host *A. altissima* (synonym *Eupatorium rugosum*), an introduced garden plant, has not yet been recorded as a weed in Australia (Randall 2007), it is known as an agricultural weed, casual alien, cultivation escape and weed in other countries (http://www.hear.org/gcw/species/ageratina_altissima/). *Ageratina altissima* is also listed as a prohibited species on the ICON website of DAFF Biosecurity (http://www.aqis.gov.au/icon), specifying that this species has been assessed as posing a high risk of becoming a weed in Australia and is prohibited entry by legislation. Damage on this non-target species is most likely to occur in the field should *B. eupatorii* be released, but as far as we know this species is not widely grown in gardens in Australia.

Baeodromus eupatorii is expected to be a damaging pathogen of crofton weed in eastern Australia where it occurs. The dew produced on most nights during autumn and winter when crofton weed is actively growing, as well as the higher rainfall during that time of the year, should be conducive to severe infections by the rust. As mentioned earlier in this document, we predict that the rust will work in tandem with *P. ageratinae,* the other pathogen that causes damage on old leaves of crofton weed in Australia, and cause increased stress on plants. One of the advantages of rust fungi over insect biological control agents in general is the high number of generations that can occur and the large quantities of inoculum that can be produced during a single growing season. Under optimum conditions, *B. eupatorii* completes its life cycle on crofton weed within 3–4 weeks.

We conclude that the level of risk associated with releasing *B. eupatorii* is acceptable and that it will be a potentially effective biological control agent. We seek permission for its release in Australia.

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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 Species of the Eupatorieae tribe found in Australia

SPECIES	SYNONYMS [1]	STATUS
Adenostemma lavenia	*Adenostemma viscosum*, *Verbesina lavenia*	Native
Adenostemma macrophyllum	*Lavenia macrophylla*	Native
Ageratina adenophora	*Ageratina trapezoidea, Eupatorium adenophorum, E. glandulosum, E. trapezoideum*	Introduced and weed biocontrol target
Ageratina altissima	*Eupatorium rugosum*, *Ageratum altissimum*	Introduced
Ageratina ligustrina	*Eupatorium ligustrinum*	Introduced and naturalised
Ageratina riparia	*Eupatorium riparium*	Introduced and weed
Ageratum conyzoides		Introduced and weed
Ageratum houstonianum	*Ageratum mexicanum*	Introduced and weed
Ayapana triplinervis	*Eupatorium ayapana*, *E. triplinerve*	Introduced
Bartlettina sordida	*Eupatorium atrorubens*, *E. raffillii*, *E. sordidum*, *Hebeclinium atrorubens*, *Neobartettia sordida*	Introduced and garden escape
Brickellia eupatorioides	*Kuhnia eupatorioides*	Introduced
Chromolaena squalida	*Eupatorium squalidum*	Introduced and eradication weed target
Chromolaena odorata	*Eupatorium odoratum*	Introduced and weed
Conoclinium coelestinum	*Eupatorium coelestinum*	Introduced
Eupatorium album		Introduced
Eupatorium cannabinum		Introduced
Eupatorium fortunei		Introduced
Eupatorium lindleyanum		Introduced
Eupatorium serotinum		Introduced and naturalised
Eutrochium purpureum var. *purpureum*	*Eupatorium purpureum*, *Eupatoriadelphus purpureus*	Introduced
Gymnocoronis spilanthoides		Introduced and weed
Liatris aspera		Introduced
Liatris graminifolia	*Lacinaria pilosa*, *Liatris dubia*, *L. graminifolia*, *L. pilosa*, *Serratula pilosa*	Introduced
Liatris punctata	*Liatris mucronata*	Introduced
Liatris pycnostachya	*Lacinaria pycnostachya*	Introduced
Liatris scariosa	*Serratula scariosa*	Introduced
Liatris spicata	*Lacinaria spicata*, *Liatris callilepis*,	Introduced

	Serratula spicata	
Mikania apiifolia		Introduced
Mikania micrantha		Introduced and weed
Mikania scandens	*Eupatorium scandens*, *Willoughbya scandens*	Introduced
Mikania ternata		Introduced
Praxelis clematidea	*Eupatorium catarium*, *E. clematideum*	Introduced and weed
Stevia caracasana		Introduced
Stevia eupatoria	*Mustelia eupatoria*, *Stevia purpurea*	Introduced and weed
Stevia lucida		Introduced
Stevia ovata		Introduced
Stevia rebaudiana	*Eupatorium rebaudianum*	Introduced

Source: Australian Virtual Herbarium (http://avh.ala.org.au/) and Randall (2007)

[1] Synonym according to the Germplasm Resources Information Network (GRIN) database (http://www.ars-grin.gov) or Australian Plant Name Index (http://www.anbg.gov.au/apni/).

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