

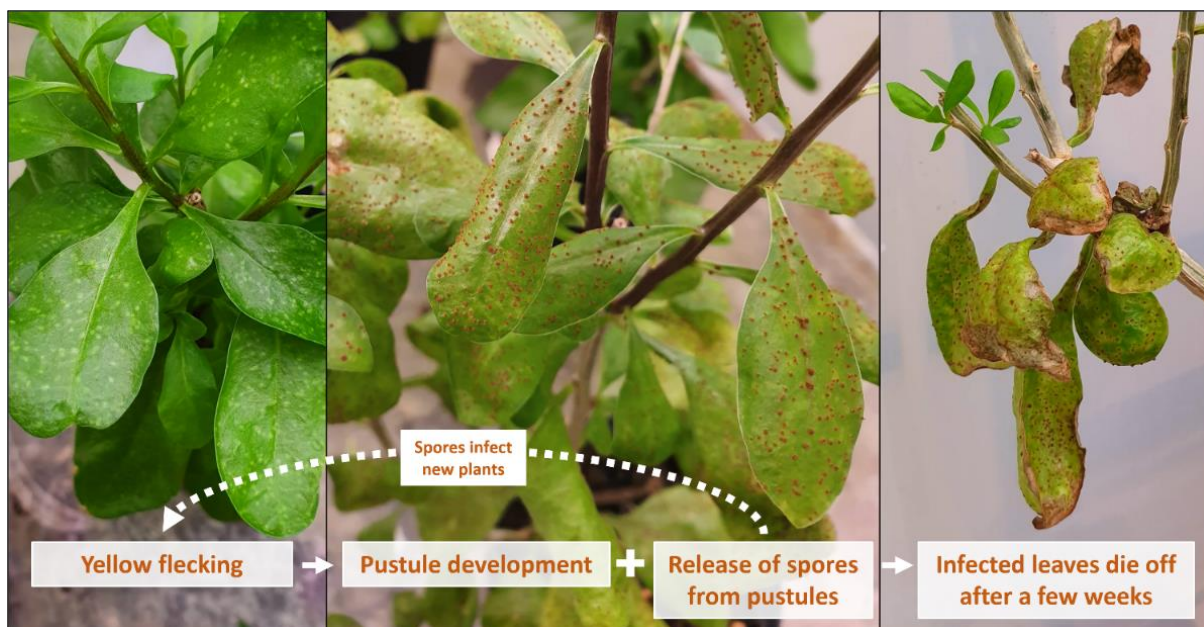
Guidelines for field releases of the African boxthorn biocontrol agent, the rust fungus *Puccinia rapipes*

Background information on the biocontrol agent

The biocontrol agent is a rust fungus, *Puccinia rapipes*, that infects the leaves of African boxthorn. It was originally isolated from diseased boxthorn plants in South Africa. Through extensive host-specificity studies undertaken by the CSIRO, the fungus was shown to be highly specific to African boxthorn and poses no danger to native Australian vegetation. In 2021, the fungus was approved for release into the Australian environment as a biocontrol agent to assist with the control of African boxthorn.

The rust fungus infects young leaves of African boxthorn, causing yellowing of the leaves followed by the development of pustules. The pustules produce fungal spores which are dispersed by wind. The spores land on the leaves of nearby African boxthorn plants and, under humid conditions, will germinate and infect new leaves. Infected leaves will die back over time. This may result in extensive defoliation of an individual plant if the fungus establishes widely and causes severe disease. Infection by the rust fungus can also disrupt the photosynthetic capacity of the plant, reducing overall plant growth and reproductive output.

Further information: <https://research.csiro.au/african-boxthorn/>



The natural infection process of the biocontrol agent.

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FOR FURTHER INFORMATION

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Release methods

CSIRO will provide participants with biocontrol agent release kit(s) that contains a vial of the rust fungus' spores, along with the materials to prepare and apply the spores to the target African boxthorn plants. One biocontrol "kit" will contain enough material to spray 8 or more boxthorn branches. If you intend to release the fungus multiple times with multiple kits, ensure that each kit released is separated by at least 200 m to enable broadscale distribution of the fungus in the local landscape.

Biocontrol agent release kit

In the release kit, CSIRO will provide you with:

- A vial containing dried spores of the rust fungus (labelled **Tube A**).
- A 25 ml plastic screwed-cap tube containing a few droplets of the surfactant TWEEN 80 (labelled **Tube B**). The non-hazardous surfactant is necessary to ensure that the fungal spores adhere to the leaves.
- A 250 ml spray bottle and spray nozzle.
- 8 plastic bags (note: to reduce plastic waste, participants will only receive a maximum of 16 bags for multiple release kits; bags can be reused for the remaining releases)

You will need to obtain:

- Approx. 250 ml tap water (if low in chlorine) or bottled water.
- Tags or bright flagging tape to mark the branches that have been treated with the biocontrol agent.
- Extra plastic bags should you choose to treat more boxthorn stems (avoid using black bags as this can cause excess heat to build up within the bag and damage the fungal spores and the plant itself).

Preparing to release the biocontrol agent

- Once you have received the release kit, ensure **Tube A** (containing the spores) is **stored in the freezer** until ready for use for up to a month.
- Plan to use the biocontrol agent late in the afternoon, preferably close to dusk and allow at least one hour to complete the release. Making the release in the late afternoon will reduce the time that the spores of the agent are exposed to the sun (and strong UV which can damage the spores), increasing the likelihood of infection of the African boxthorn plants.

The biocontrol agent should be released in areas with the following attributes and conditions:

- **Dense infestations of African boxthorn**, to increase the opportunities for other leaves and plants to become infected by the fungus.
- On **healthy, juvenile plants or mature plants with fresh growth**. The fungus has a strong preference for new growth over old growth.
- In areas where the African boxthorn population will **not** be managed by the application of chemical herbicides, slashing or other control methods.

It is your responsibility to ensure that you have obtained permission from the relevant landowner/land manager/custodian to release the African boxthorn biocontrol agent at the nominated site.

Releasing the biocontrol agent

1. Fill **Tube B** with water up to top of the container, close with the lid provided, and gently swirl the tube to mix the TWEEN and water. Pour **Tube B** into the **spray bottle** and fill up the spray bottle with water to the top mark (250 ml total).
2. Add the spores from **Tube A** into the spray bottle and gently shake or swirl the bottle until the spores are mixed into the TWEEN solution. Do not shake the bottle too vigorously as this can cause the TWEEN to bubble. **Please use the spore solution within 2 hours of making it up.**
3. Tag each sprayed stem with flagging tape or any other markers/tags you wish to use to aid identifying the fungus when monitoring for infection success in the future. Remember that each 250 ml bottle will be enough to spray up to **8 branches across 2 or more plants** – if you have excess solution, you can spray additional stems, if you have extra bags to cover them.
4. Spray the tagged boxthorn branches with the spore solution until droplets form on the leaves. Ensure to spray both the top and underside of leaves and the branch. Once a branch has been sprayed, cover with plastic bags provided, and tie the ends of the bag together to hold it fast to the branch.
5. Complete the **baseline monitoring datasheet** (last two pages) and return the sheet via email at your earliest convenience to boxthornbiocontrol@csiro.au.
6. Leave each treated branch covered overnight. Revisit the field site the following **morning** to remove each bag and to take the following photos (photo examples below):



(a) **Sprayed stem** – a close-up photo of one or several of the tagged stems that were sprayed, as a record of the quality of the leaves prior to applying the biocontrol agent. (b) **Individual plant** – a close-up photo of the African boxthorn plant(s) that were infected, as a record of plant health condition. (c) **Landscape** – depicting the condition of the African boxthorn habitat.

Monitoring and evaluation

Baseline monitoring

In return for receiving the African biocontrol agent, we would be grateful if you could complete the baseline monitoring datasheet at the end of this document. Please send a copy of the completed datasheet via email to boxthornbiocontrol@csiro.au at your earliest convenience.

We will use this information for monitoring and evaluation research purposes, and to understand the environmental determinants of establishment and spread of the biocontrol agent. All personal information will be treated confidentially in accordance with CSIRO's Human Ethics protocols (see participant information sheet provided).

Follow-up monitoring

We also request that you return to the release site after approximately 6-8 weeks and take a photo of each stem to which the biocontrol agent was applied. This will help us to identify the presence or absence of the biocontrol agent, which produces characteristic dark brown pustules. See photo below for examples.



Baseline monitoring datasheet

for *Puccinia rapipes*, a biocontrol agent for African boxthorn.

Please complete the following datasheet while you make your releases of the biological control agent. Please scan or take a photo of the completed datasheet and email the datasheet and site photos to the CSIRO boxthorn biocontrol research team at boxthornbiocontrol@csiro.au. Thank you for your participation in the release project.

Release site information		
Date:	Name ¹ :	Affiliation (e.g. private landholder, local or state government biosecurity officer etc.):
Site location and address:		State:
Number of releases made (number of stems targeted with the agent):		
Release location GPS coordinates ² :	Release date:	Release time:
Photographs		
<p>(1) Each sprayed stem - close-up photo taken of the stems that were treated with the biocontrol agent solution to provide a record of leaf quality.</p> <p style="text-align: center;"><input type="checkbox"/> Yes <input type="checkbox"/> No</p>		
<p>(2) Individual plant - a close-up photo of the African boxthorn plant(s) that you wish to infect as a record of plant health condition.</p> <p style="text-align: center;"><input type="checkbox"/> Yes <input type="checkbox"/> No</p>		
<p>(3) Habitat - a general landscape photo depicting the condition of the African boxthorn habitat at the time of releasing the biocontrol agent.</p> <p style="text-align: center;"><input type="checkbox"/> Yes <input type="checkbox"/> No</p>		

Weather and habitat characteristics	
Rainfall	<input type="checkbox"/> No rainfall <input type="checkbox"/> Rained during the day ____mm <input type="checkbox"/> Rain previous evening ____mm <input type="checkbox"/> Rain expected overnight ____mm
Estimated temperature at the time of release	
Weather conditions at the time of release (tick all that apply)	<input type="checkbox"/> Clear <input type="checkbox"/> Cloudy <input type="checkbox"/> Calm <input type="checkbox"/> Windy <input type="checkbox"/> Dry <input type="checkbox"/> Overcast <input type="checkbox"/> Breezy Other, please list:
General description of the habitat at the release location	
African boxthorn plant status (tick all that apply)	<input type="checkbox"/> Healthy <input type="checkbox"/> Stressed <input type="checkbox"/> Fresh growth present <input type="checkbox"/> Flowers present <input type="checkbox"/> Fruit present <input type="checkbox"/> Canopy cover over plants
Demographic life stages present (tick all that apply)	<input type="checkbox"/> Seedlings (0-30 cm) <input type="checkbox"/> Juveniles (~30 -50 cm) <input type="checkbox"/> Adults (>50 cm)
Density of African boxthorn infestation	Number of plants in an estimated 20 X 20 m square quadrat: ____plants % boxthorn groundcover in estimated 20 X 20 m quadrat: ____%
Estimated area invaded by African boxthorn (hectares, 100 X 100 m)	
Any other comments, details or feedback.	

¹Personal information will be treated confidentially in accordance with CSIRO's Human Ethics protocols (see participant information sheet provided).

²How to extract GPS coordinates of a location using the Maps app on your smartphone:

Using Google Maps:

- 1) Open the Google Maps app on your smartphone or tablet.
- 2) When the blue circle for your location appears on the map, touch and hold this area of the map (that isn't labelled) to drop a red pin.
- 3) The GPS coordinates will appear in the search box at the top of the screen.

Using Apple Maps:

- 1) Open Apple Maps on you iPhone or tablet.
- 2) Tap the current location button on the top right.
- 3) When the blue circle for your location appears on the map, tap it.
- 4) Swipe up from the bottom to view full details for your location and the GPS coordinates will be listed for your location.