



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

This new project is funded by the **Australian Government's Future Drought Fund** and delivered by CSIRO under the national 'Nation-wide Weed Biocontrol Mass-Rearing and Release Network for Enhanced Drought Resilience in Australia's Agricultural Landscapes' led by the Centre for Invasive Species Solutions.

For more information on the African boxthorn biocontrol agent, please visit: <https://research.csiro.au/african-boxthorn/>

Important! Before making your biocontrol releases:

- Once you have received the release kit, ensure **Tube A** (a very small vial containing the spores) is **stored in the freezer** until ready for use for up to 2 months. After 2 months, spores will start to lose their viability.
- **Plan to release the biocontrol agent as late in the day as possible**, 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 fungal spores are exposed to UV which can damage the spores.
- The target boxthorn plants **must** have healthy, green leaves present – this fungus infects the leaves and green parts of fruit/flowers. It will not establish on woody stems.
- The biocontrol agent should also be released in areas with the following attributes and conditions:
 - In **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.
 - Areas close to water (i.e.. rivers, dams) can improve the success of the biocontrol agent – the higher humidity around these areas creates more ideal conditions for the fungus to persist, especially during dry summers.
- **Your safety is your responsibility.**
- **It is your responsibility to ensure that you have also obtained permission from the relevant land manager or custodian to release the African boxthorn biocontrol agent at the nominated site.**

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.

The biocontrol agent release kit

In the release kit, we will provide you with:

- A small 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 between each release)

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).

Releasing the biocontrol agent

1. Pour the contents of **Tube B** into the spray bottle and fill up the spray bottle with water to the top mark (250 ml total). Give a gentle swirl to mix the TWEEN.
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 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. Aim to apply the fungus to **one branch per plant**.
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.
5. Remember that each 250 ml bottle will be enough to spray up approx. **8 branches across 8 plants** – if you have excess solution, you can spray additional stems/plants if you have extra bags to cover them.
6. 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.
7. 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).
8. Complete the **baseline monitoring datasheet (pages 4 and 5)** and return the sheet via email at your earliest convenience to boxthornbiocontrol@csiro.au.



Monitoring and evaluation

Baseline monitoring

In return for receiving the African biocontrol agent, participants are requested to complete the baseline monitoring datasheet on pages **4 and 5**. Please send a copy of the completed datasheet via email to boxthornbiocontrol@csiro.au or by post to: **Caroline Delaisse, GPO Box 1700, Canberra ACT 2601**.

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 information collected will be treated confidentially in accordance with CSIRO's Human Ethics protocols (see **Research Participant Information Sheet provided** in previous emails).

Follow-up monitoring

Participants are encouraged to 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 photos below for examples of infection.

You can complete the table at the end of this document on **page 6** and email or post it back to us.



Biocontrol agent infection of stems and leaves. Stem lesions are more common and easily observed than leaf lesions

Baseline monitoring data sheet

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 African boxthorn biocontrol research team at boxthornbiocontrol@csiro.au. Thank you for your participation in the release project.

1. Release site information			
Name(s):			
Release location GPS coordinates:	Release date/time:	Number of kits released per site	Number of plants treated per site
Photographs (with examples)			
<p>(1) 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>(2) Photo point of the African boxthorn infestation: a habitat scale photo depicting the condition of the African boxthorn infestation and surrounding vegetation at the time of inoculation. You may wish to select a point that you can return to and take photos of the site over time to track visual changes over the course of the trial (photo point monitoring).</p> <p style="text-align: center;"><input type="checkbox"/> Yes <input type="checkbox"/> No</p>			
 <p style="text-align: center;">Photo of target plant</p>		 <p style="text-align: center;">Photo of site and infestation</p>	

Weather and habitat characteristics	
Rainfall (tick all that apply)	<input type="checkbox"/> No rainfall <input type="checkbox"/> Rained during the day <input type="checkbox"/> Rain previous evening <input type="checkbox"/> Rain expected overnight
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:
African boxthorn plant status (tick all that apply)	<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)
Estimated % foliage cover of the target plants	
Estimated % of the site invaded by African boxthorn (e.g. 20 % cover of 1 ha area)	
Any other comments, details or feedback.	

How 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.

2. Post-release monitoring (Complete this any time after 8 weeks post-biocontrol agent releases and/or in spring)

Name(s):

Release location GPS coordinates:	Observation date	Infection present? (Yes/No)	Out of the stems that were treated, how many were infected?

Photos taken of:
 Individual plant Yes No
 Habitat Yes No

Any additional observations or notes?