

Arabidopsis thaliana Guidance Document:

This document is intended to help establish best practice policies when containing the HSNO approved organism *Arabidopsis thaliana* – to be developed or imported. This is guidance to meet the controls imposed by the approval ERMA200706 and ERMA200792.

Controls

The <approval user> must ensure compliance with the controls set out below in respect of any work carried out under this approval.

1. The approved organism (Table 1) must be held in containment.
2. Development conducted under this approval must be conducted within the containment facilities listed in Appendix 2
3. This approval is limited to the development in containment of *A. thaliana* whole plants and heritable material for the purposes of laboratory-based contained research.

Guidance Note: This includes cell or tissue culture or whole plants that may form reproductive structures. Seeds are heritable material. Because *A. thaliana* is self-pollinating, extra consideration for containment of pollen is not warranted.

Requirements for containment facility

4. The containment facility must be designed and constructed to contain the approved organism held within it under any reasonably foreseeable circumstances.

Guidance Note: The Australia/New Zealand Standard: Safety in Laboratories 2243.3 and AS/NZS 2982 Laboratory Design and Construction are both invaluable resources for design assistance. It is strongly recommended that advice should be sought from MAF at the time of construction. MAF should be informed at all stages of design and construction, and at the time of any alterations to the facility.

In light of the organism to be contained, it is the seed that has the highest potential risk of escape, therefore we have highlighted structural elements designed to reduce the potential of seed to escape the facility:

- **The junction between floor and wall in containment glass houses/facilities should be sealed.** This might be achieved a number of ways, including by a sealed concrete nib wall which is mortared to floor pad, or in the case of internal plant facilities by covered flooring.
- **There should not be any gaps under doors.** This could be achieved through bunding, or a raised door entrance to prevent seed being blown under the door. Otherwise the door could be fitted with brushes, but attention should be given to the capture of seed in the unlikely event that seed makes its way through the brush barrier.
- **Where drains are present care is needed to prevent seed being washed down the drain. It is also important that drains do not provide a point of entry for rodents and insects.** It is suggested that drains are screened with fine mesh (the size of which should reflect the size of *A. thaliana* seed), or a wad of filtration material (such as F6 air filtration matrix) that can be removed when clogged. This filtration material can be autoclaved, disposed of, and easily replaced. Regular checks of the mesh and/or filtration material should be carried out as part of routine maintenance.
- **All waste water should be managed to ensure it contains no live approved organisms or heritable material.** Water management practices may include the use of disinfectants for small volumes of water (up to 50 litres) to render any seeds non-viable (note: disinfectants will need to be very aggressive to render the seed non-viable), or filtering to prevent the escape of any live plant material, taking in consideration of the size of seeds. The water should then be discharged into municipal sewer systems in sealed pipes with no open access to the external environment, where it will undergo further treatment before release. Filters should routinely be changed and waste material disposed of as per approved waste disposal procedures.
- **A freestanding containment facility should have an anteroom for entry and exit. An anteroom is not necessary if the facility connects directly to a certified containment facility.** Facilities may consider a positive air pressure anteroom, to ensure any seed will be blown back into the containment facility. This will also prevent easy entry of unwanted contaminants and insects.
- **Flooring material should be smooth, seamless, and impervious to allow easy capture of seed.** It is suggested that the use of a vacuum cleaner (fitted with a HEPA filter) can reduce or remove the need to use water on floors.
- **Any structural modifications made to the containment facility must be approved by a MAF inspector prior to being used.**

Note: Notwithstanding the need for proper containment facilities, the primary emphasis on containment of *A. thaliana* seed must be on seed capture by primary and secondary containers (ie bagging of seed heads and large trays).

5. The containment facility must be maintained in order to contain all approved organisms held within it (i.e., preventing escape) under reasonably foreseeable circumstances.

Guidance Note: This control refers to the physical maintenance of the containment facility. All regular users of the facility should be instructed to recognise and report problems.

- **It is highly recommended that a dedicated person be appointed, and a regular schedule should be in place to monitor the facility, and to check/clean filters, and perform other necessary maintenance.**
- **All users should be trained to recognise and immediately report problems or potential problems that may result in a breach of containment.**

6. All reasonably practicable measures must be taken to prevent the accidental or deliberate release of an approved organism from the containment facility.

Guidance Note: This control reflects the procedural policies that must be in place to contain *Arabidopsis* in the facility.

- **It is recommended that all plants housed in the plant house should be considered and treated as new organisms; therefore, containment policies and should be implemented for all plants in the facility.**
- **A central register is highly recommended. This should demonstrate that an organism is part of the HSNO approval, and will allow tracking of organism (plant/seed) provenance.**
- **The use of easily cleaned large trays under plants (including at sowing stage) is crucial. The trays should be large enough to extend well beyond the spread of the plant to ensure that any seed falling from bent stems will be captured.**
- **Bagging of seed heads (or growing in high sided containers) is an a important primary containment measure, but plants should also be on larger trays which act as secondary containment and prevent seed falling to the floor where is it less likely to be contained. Note: Containment of seed is the most important primary containment measure. However, if bagging of seed heads is premature, there is a risk of fungal infection resulting in loss of important research material and promoting over-planting. It is recommended that bagging (or a similar measure) is undertaken when the siliques turn yellow. Plant house staff should be instructed to**

bag plants where necessary to ensure the seed head will always be bagged. Alternatively, the plants can be grown in high sided containers to prevent seed falling onto the floor from seed heads.

- **Dedicated laboratory coats for use only in the plant house are required. It is preferable that these lab coats should be pocketless. It is also recommended that disposable shoe covers or dedicated shoes should be worn in the plant house (option) to prevent the escape of seed.**
- **Sticky floor mats supplied for clean rooms are helpful, especially for indoor facilities.**
- **Dedicated wet and dry vacuum cleaners are also helpful. These should be fitted with a HEPA filter, and the waste contents from the vacuum cleaner must be autoclaved before disposal.**
- **It is suggested that containment protocols are reviewed annually, and that all technical and research staff are involved in the review. This ensures all containment protocols are current, workable and take into account any incidents that indicate potential failure of containment measures.**
- **Users should consider the use of artificial growth media. Artificial media is lighter, does not harbour mites, is more easily transported, and more easily autoclaved and disposed of than soil.**

Requirement for entering/exiting containment facility

7. All reasonably practical measures must be taken to ensure that persons entering and exiting the containment facility do so in a way that does not compromise the containment of the approved organism.

Guidance note:

Procedures should be in place to ensure all users of the facility (including any new or short-term users) are aware of the controls required by this approval.

- **All users must be trained in the procedures required by this approval.** It is recommended that the facility manager should ensure that only those persons who have bona fide scientific research requirements and who have been properly instructed are permitted access to the facility. Management staff should not be given default access unless they are properly instructed and are aware of containment measures.

- **Contractors, trades people, and visitors may only be given access to the facility after adequate instruction.** It is suggested that visiting trades people should be required to sign a simple sheet indicating they are aware that the plant facility is for the containment of plants and seeds, and they will observe all entry/exit procedures, will not come in contact with plants, and will ask trained personnel to move plants if required. Trades people should also be instructed to inform the plant house technician when work has been completed and their exit should be documented.
- **Maintenance staff should be instructed in case of emergency.** The tradesperson induction should include a requirement to contact the plant house technician in the event of an emergency.

Requirements for moving new organisms

8. All reasonably practicable measures must be taken to prevent the escape of an approved organism during any transfers within the containment facility or outside the containment facility.

Guidance Note:

- **Double containment is required for transport between the plant facility and laboratories.** Any approved organism must be contained in at least two containers for transportation. It is suggested that these could include plastic microcentrifuge tubes, plastic screw-top centrifuge tubes, or air tight plastic boxes.
- **Three layers of containment are recommended for transport outside of the containment facility.**

9. An approved organism may only be removed from a containment facility for a reasonably necessary purpose (e.g. transfer to another containment facility).

Guidance Note:

- **In the specific case of moving saved seeds to another containment facility for storage.** Where saved seeds are moved for storage purposes, they must be stored in another containment facility and transported inside double containers (see above). When stored, seeds should be maintained in a spill-proof, labeled container. Seed containers must clearly identify the provenance of the seed and its genetic background.

10. Containment measures for approved organisms being transferred must clearly identify the contents, containment requirements, and the details of the sender and receiver.

Guidance Note: Stored seed containers must clearly identify the provenance of the seed and its genetic background.

- **A log of all new organisms imported into, transferred out of, or produced within the facility must be kept.** This log must describe the new organism, and provide the HSNO Act import or development approval number, its location within the facility, or if it has been disposed of or transferred to another containment facility. It is sufficient to describe the organism at the experiment level.

Requirements to limit access to the containment facility

11. All entrances must clearly identify the facility as being a containment facility.

Guidance Note: Signs should be put up at all laboratory entrances indicating the area is a containment facility and access is limited to authorised personnel.

- **It is suggested that in addition to standard containment facility signage the additional requirements of a restricted plant house should be displayed.** These should include contact mobile telephone numbers for responsible persons, and requirements for trades people and facilities management staff to contact the plant house technician (see Guidance note for control 7).

12. All entrances into the containment facilities must be specified.

Guidance Note:

- **Floor plans are the clearest method to show where all the entrances are into the containment facility. Floor plans must be kept up to date.**

13. All reasonably practicable measures must be taken to prevent unauthorised persons gaining access to the containment facility.

Guidance Note:

- **All doors should be lockable and locked when not in active use.**
- **Contractors and trades people should only be given access after receiving adequate instruction. See guidance note for control 7.**
- **Management staff should not be given default access to the facility unless they are properly instructed and aware of containment measures.**

Requirements for removing equipment and waste from the containment facility

14. Any waste (including biological material) that may contain an approved organism, or heritable material from an approved organism, must be treated to ensure that the approved organisms and any heritable material is killed prior to its removal from containment.

Guidance Note:

- **Autoclaving is the preferred method of treatment to kill or neutralize waste or heritable material from an approved organism.** Other treatment methods may include the use of chemicals or incineration. Researchers should consider using artificial growth medium instead of soil when growing *A. thaliana* plants. Medium is much lighter and more easily contained than soil, making transport to and from the autoclave less problematic.

15. Any equipment that may harbour the approved organism, or heritable material from the approved organism must be treated to ensure that the approved organism and any heritable material is killed prior to being used for another purpose or removed from the containment facility.

Guidance Note: Treatment of equipment to remove any approved organisms or heritable material can include treatment of any parts of the equipment that come in contact with the approved organism or heritable material, and can effectively be treated.

Training

16. All persons entering the containment facility (including contractors, staff, students, visitors and volunteers) must have received instruction on the containment practices of the containment facility relevant to the responsibility of the individual.

Guidance Note:

- **All training should be documented.** All staff, students, and researchers working within the facility must be instructed on the operational procedures for containment. All tradesmen, contractors, visitors and volunteers entering the facility must be instructed on containment procedures, relative to their responsibility. See guidance note on contractors above.

- **Ongoing refresher training is critical.** Regular users of the facility should be involved in regular periodic reviews of containment procedures and required to report any potential breach of containment.
- **Training should include (but is not limited to):**
 - HSNO controls on the approved organism
 - Possible pathways of escape of the approved organism, and the procedures in place to maintain containment.
 - Organisational risk management associated with maintaining containment
 - Contingency plans.

Requirements for contingency plans

17. The containment facility must have a documented contingency plan detailing processes on the event of a breach of containment, including recapture or eradication protocols, for each approved organism held within the containment facility under this approval. The contingency plan must cover any reasonably foreseeable event that could compromise containment of any approved organism within the containment facility, and the plan must be capable of implementation.

Guidance Note:

- **Contingency plans should be a part of any training that occurs to educate staff on policies.** Contingency plans may include a regular herbicide spraying regimen around exterior plant houses, regardless of any breaches.
- **Discussion with your MAF inspector on follow up procedures is important.**
- **Plans should also reflect when a decision should be made to destroy the plants to guarantee it will not escape.**
- **It is recommended that your contingency plans take into consideration that many emergencies will affect communications and electricity supply.** This may not happen right away but some time after the emergency (eg. Cell phone towers have a short battery life). Provided the structural integrity of the facility is intact, a contingency plan may include closure of the plant house to prevent entry until essential services are returned to normal.

18. The contingency plan must be implemented if there is reason to believe that a new organism has escaped or been released from the containment facility.

Guidance Note:

- **The facility must have documented contingency plans that are to be implemented should there be a suspected release or escape from containment.** The facility must hold the means to implement the contingency plan, including but not limited to an effective herbicide.

Requirements for inspections and monitoring

19. To ensure containment is being achieved and to identify any remedial maintenance requirements each containment facility must be inspected at reasonable intervals given the nature of the approved organism(s) being contained.

Guidance Note:

- **Regular maintenance programs should be developed, including internal audits, and routine maintenance checks, etc.** The responsibility is on the organisation to ensure internal audits are conducted regularly so that any faults in containment are remedied as soon as possible. All users of the plant house should be involved in surveillance through the reporting of faults or potential problems.

20. MAF must be allowed access to the containment facility and relevant documentation for the purpose of auditing.

21. Each containment facility must be inspected as soon as possible after any event that could compromise the containment regime such as an Act of God (such as flood, earthquake), or any unauthorised attempt to enter the containment facility.

Guidance Note:

- **Keep in regular contact with your MAF inspector as soon as possible to update them on conditions and status.**

22. Faults in containment must be remedied as soon as possible, including taking such interim measures as are necessary to mitigate the risk of breach of containment.

Guidance Note: This might mean approved organisms may need to be moved to a separate area within the containment facility, or to another containment facility.

- **Saved seeds may be moved to another containment area on the same institutional site for storage.** Seeds should be maintained in a spill-proof, labeled container.
- **This might mean increased inspection rates, or spraying of herbicide, as outlined in the contingency plan.**

23. Any structural modifications to a containment facility must be approved by a MAF Inspector prior to being used to contain the approved organisms.

Restrictions on Genetic Modification

The applications for *Arabidopsis thaliana* import and development state that modifications to *A. thaliana* will not involve:

- the production of infectious particles
- genetic material derived from Māori
- genetic material derived from native flora and fauna
- genes encoding known or predicted vertebrate toxins
- uncharacterized sequences from pathogenic microorganisms
- genetic modifications that increase the pathogenicity, virulence, or infectivity of the host organism
- genetic modifications that result in the modified organism having a greater ability to escape from containment than the unmodified host.

Guidance Note: These restrictions are imposed to ensure the modifications to *A. thaliana* under these import and development approvals are consistent with the HSNO Low-Risk Genetic Modification Regulations 2003. If you plan to develop or import *A. thaliana* with modifications that fall outside these exclusions, or which are ambiguous, you may require a separate HSNO application. For further clarification you should consult with the EPA in the first instance (email: noinfo@epa.govt.nz).

- The term 'unmodified host' refers specifically to the original wild-type strain of *A. thaliana*; any genetic modifications must not result in the modified strain having a greater ability to escape containment than wild-type *A. thaliana*. For example, gene complementation experiments are permitted.

- Care should be taken when planning experiments to test altered growth, productivity, and reproduction of Arabidopsis. When planning these types of experiments, due consideration should be given to the expected effects. For example, modifications that increase seed production are not likely to increase the risk of escape; however, modifications that reduce seed size are likely to give the organism a greater ability to escape, and specific protocols for the containment of seed may need updating. For further clarification on whether a proposed modification complies with these approvals, please consult the EPA (see above).