Recommendations on Critical Infrastructure
Kenya (Njoro) and Ethiopia (Ambo, Debre Zeit and Kulumsa)

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Background

Appropriate facilities are needed for wheat rust research. Although there are different requirements depending on the objectives of the work, e.g. detailed race analyses vs. routine screening of breeding lines, a basic infrastructure is needed. Critical infrastructure includes the following:

- Seedling growth facility. Ideally this area should be isolated from rust spores and air movement that may disseminate spores to plants not yet inoculated. This facility is essential for race analysis but not for general screening where race mixtures are often used.
- Inoculation booth with turntable and flushing system. Depending on the pot type and size used, a tray containing a differential set will usually be placed on a turntable within the inoculation booth. The turntable could be motorized but this is not essential. It is important that the entire booth can be flushed with a water spray between isolates. This requires a cover. The booth needs to be custom built and the final design and size will depend on the space available and requirements of the scientist:

- Inoculators and spore collectors. Many types of inoculators are available. For race analysis it is suggested to use a system similar to that of the CDL in St Paul. This system consists of small hand-held inoculators and cyclone collectors. A gelatin capsule or other type of tube for holding the inoculum is interchangeable between the two devices. These items have to be custom built. The material from which they are made should allow alcohol and/or acetone washes as well as sterilization in an oven. The same system could be used for inoculation seedlings of breeding lines and in some cases modifications are made to accommodate larger volumes of inoculum. If gelatin capsules are used, spores cannot be dispersed in a water suspension. To connect the inoculator and collector to the pressure or vacuum line a special T-piece is needed.

A. Spore collectors, B. Inoculator, C. Inoculation

- Vacuum/pressure pump. A laboratory pump is required for collecting spores and atomizing them onto seedlings. In selecting a pump care should be taken to allow for sufficient pressure. Depending on the layout of the facilities, it is advisable to have one pump for collection and one for inoculation.
- Tubes/capsules. Eppendorf tubes or capsules could be used. The CDL system use size 00 gelatin capsules of 1 ml capacity.
- Oil. A light mineral oil is required to suspend the spores in the capsules or tubes. An example is Soltrol-130.
- Dew chamber. An efficient incubation chamber for exposing plants to a dew period is essential for rust research. The system should allow dew to be formed on leaves while maintaining an appropriate temperature window for the respective pathogen. Many types of dew chambers are used. The simplest form is spraying plants with $dH_2$0 after inoculation and placing them in a plastic bag or larger container sealed with a plastic or other type of cover. A wet towel could be placed on the inside of the cover and it is advisable to add water to the bottom of the chamber for maintaining saturated conditions. Other incubation systems include chambers constructed of perspex or galvanized metal sheeting. Dew can be created by operating ultra-sonic humidifiers or running water baths in the bottom of the chambers. Again, the efficiency of the system is a function of chamber size, ambient temperature and frequency and duration of humidification. Larger labs have walk-in rooms with temperature and humidity control. Dew chambers are available commercially but they are expensive. It is recommended that chambers are constructed locally according to available space and needs.

Example of a dew chamber used at the Plant Breeding Institute, Australia

- Drying-off area. An area is needed where plants can be placed to dry off after inoculation (evaporation of oil) and to acclimatize after removal from the dew chamber. At this stage leaves and initial infections are sensitive to rapid changes in temperature. The area where plants are placed after inoculation has to be free of any contaminant spores, either from plants elsewhere in the greenhouse or from subsequent inoculations.
- Single-pustule management facility. For purifying races a system is needed where seedlings bearing only one pustule can be isolated and re-incubated. Typically this could be done by placing 2-L plastic soft drink bottles over the pot in a water filled tray, or any other tubing/cellophane-covered device.
- Water bath. Needed for heat shocking of spores after retrieval from the freezer. Needs to maintain temperature in 45-50°C range. This can be achieved using hot tap water with temperature adjusted accordingly, if cost precludes purchasing a water bath.
- Sterilizing oven. For sterilizing inoculators and collectors (if constructed from aluminium or stainless steel).
- Microwave oven. For drying silica gel.
- Greenhouse compartments with reasonable temperature control (15-25°C).
- Sensors for measuring temperature. Electronic data loggers (*e.g.* Tiny Tags) are required for each greenhouse room as well as for the dew chamber. Tiny Tags data loggers are available for AUD$220.00 per unit, with software and cables costing AUD$190.00. These units are weather proof, can be programmed to take regular measurements of temperature (if set to capture readings at 15 minute intervals, they can record for 9 to 12 months), and will record within the range of -40° to +85°C. Data is downloaded to Excel and is easy to manage.
- Minus 80°C freezer, racks (filing system) and cryotubes. Essential for long-term storage of isolates. Freezers are available commercially. Freezer should be placed in a well-aired area to allow optimal functioning of the compressors. Back-up cultures of the most important races have to be stored in a different freezer at all times. This could be at other research stations.
- Refrigerator. For keeping cultures for short-term storage. Many races could be stored in light mineral oil in a refrigerator for 2-3 months.

- Seed storage facility. A facility that will allow storage of germplasm collections (i.e. differential sets) is required. High humidity and excessive temperatures should be avoided.
- Maintenance. Specifically for greenhouse air conditioning and -80°C freezer.
- Back-up generator. Essential during power cuts for operating a greenhouse and other critical equipment such as the freezer.
- Pots, storage and cleaning facility. Plastic pots should be used as this allows easy cleaning and storage. A cleaning facility, usually a large container to which a chlorine solution can be added, will suffice.
- Disposal system for rusted plants. Plastic bins with lids to avoid disposed plants continuing to serve as sources of contaminant spores.
- General lab consumables, *e.g.*
    - Pipettes / dispenser (for oil)
    - Glassware (desiccators, beakers, McCartney bottles, Shott bottles etc)
    - Thermometers
    - Maleic hydrazide
    - Pot labels
    - Marker pens
    - Potting mix (good quality) plus undercover storage facility for mix
    - Fertilizer, preferably a liquid formulation
- Field inoculator. Ultra-low-volume applicator (manufacturer, Microfit®, Micron Sprayer Ltd., Bromyard, Herefordshire, UK) should be purchased to allow field rust inoculations:

Greenhouse, laboratory and field infrastructure was inspected at KARI Njoro Kenya and EIAR Ambo, Debre Zeit and Kulumsa Ethiopia in June 2008. It was recognised that in most if not all cases considerable value could be obtained by contacting local horticultural industries to see what materials are used in greenhouse/ shadehouse construction, pots and potting mix etc. Several proposals were developed for each site, to ensure the most efficient use of the funding available. Each proposal is detailed below.

KARI Njoro Kenya June 8-11

Backup generator power is provided to all facilities in the advent of a power black-out. However, concerns were expressed about the cost of running the generator, which uses considerable quantities of diesel fuel.

Laboratory infrastructure

The plant pathology laboratory at Njoro, although not fully equipped, has excellent space and benches. The most important things needed to allow preservation of rust isolates are:

- minus 80°C refrigerator
- microwave oven (for drying silca gel)
- desiccators to allow drying of rust spores
- Eppendorf tubes (cryovials) with screw lids

A microscope has been requested to allow spore viability to be checked. There are relatively cheap (<$500) recently developed digital compound microscope that work from a USB connection on a computer that could suffice for this purpose. These microscopes also allow image capture. I will look into this and report back on suitability and cost. If funds will allow, an existing freeze drying unit could be refurbished (I understand all that is needed is a vacuum pump) to allow back-up isolates to be established for each pathotype preserved. Such back-up isolates could be stored independently in a normal refrigerator, in another building, to safeguard against loss of isolates in the case of fire.

Greenhouse infrastructure

The greenhouse facilities were established in the late 1960s and are considerably run down. They comprise a large greenhouse with four sections (“Main greenhouse”; Appendices I-1A. I-1B, I-1C, I-1D), several small greenhouses (“Small greenhouses”; Appendix I-3A), two quite dilapidated buildings in which stripe rust work was once conducted (there are plans to demolish these; “Former stripe rust facility”; Appendices I-3A, I-3B), and a building comprising 24 small rooms in which presumably rust isolates were maintained in isolation to ensure purity (“Rust isolation facility”; Appendices I-2A, I-2B).

There is no functional temperature control in any of these facilities. We were advised by Dr Don Harder, formerly from Agriculture Canada and who worked on stem rust at Njoro in the early 1970s, that the “Main greenhouse” was used for stem rust work and at times for leaf rust work, and that it was cooled by circulation of outside air only. He also indicated that the “Small greenhouses” were also used for rust work, and that he also used the “Former stripe rust facility". He did not use the "Rust isolation facility", and indicated that this must have been built after his time there.

As refurbishing greenhouse infrastructure will be the most costly, we have proposed three alternatives that are anticipated will be incrementally more expensive and of which the second is preferred. Items of equipment/ repairs needed that are common to all three are:

- routine maintenance to make venting system (side walls and roof apex) operational again
- reconditioning cooling system for main greenhouse
- "Tiny Tag" temperature data loggers for temperature monitoring, with OTLM software

<u>Proposal 1.</u>

a. Refurbishment of "Main greenhouse" (Appendix I-1C)
b. Alteration to "Rust isolation facility" (Appendix I-2C)

The "Main greenhouse" would be upgraded and modified by:

- Refitting air circulation system and possibly adding a wet wall in front of air intake.
- Installing retractable internal heat blankets in compartments 4a and 4b.
- Constructing two longitudinal walls (from cement sheeting to waist height, polycarbonate above) to separate compartments 4a and 4b. A door to be installed in each wall to allow access to each compartment.
- Benches to be installed in compartments 4a and 4b.
- Healthy seedlings to be raised in compartments 1a and 1b.
- Infected plants would be maintained in compartments 4a and 4b.

The "Rust isolation facility" would serve as an area in which inoculations are conducted and plants given a dew treatment to induce infection. This would require removal of a wall to combine two isolation rooms, to provide an area for inoculation. This room would be fitted with sprinklers to allow it to be washed out between inoculations. A room opposite this could be fitted with an ultrasonic humidifier and be used to incubate.

<u>Proposal 2 (preferred)</u>

Development of "Main greenhouse" to permit all steps involved in raising, inoculating and assessing rust response (Appendix I-1D).

The "Main greenhouse" would be upgraded and modified by:

- Refitting air circulation system and possibly adding a wet wall in front of air intake.
- Installing retractable internal heat blankets in compartments 1a, 1b, 2a, 2b, 3a and 3b.
- Constructing two longitudinal walls (from cement sheeting to waist height, polycarbonate above) to separate compartments 1a, 1b, 2a, 2b, 3a and 3b. A door to be installed in each compartment wall to allow access.
- Benches to be installed in compartments 1b, 2a, 2b, 3a and 3b.

➢ Healthy seedlings to be raised in compartment 1b.
➢ Inoculations to be conducted in compartment 1a.
➢ Infected plants would maintained in compartments 2a, 2b, 3a and 3b. One or more of these compartments could have supplementary air conditioning fitted at a later date to allow work with stripe rust.

Compartment 1b would be developed as an inoculation area, requiring an inoculation chamber to be built and installed on the sink (water supplied from taps and water drained to sink). An incubation (dew) chamber would be built and installed as shown. This chamber would be built from insulated material, have temperature control (*ca*. 12–23°C), and have either a thermostatically controlled heated water bath to generate dew, ultrasonic humidifiers (x3), or trays that can be filled with water and covered with hoods.

<u>Proposal 3</u>

a. Refurbishment of “Main greenhouse” (Appendix I-1C)
b. Reconstruction of “Former stripe rust facility” to allow rust inoculation and incubation.

The “Main greenhouse” would be upgraded, modified and used as in Proposal 1.

The “Former stripe rust facility” would be refurbished by:

- Replacing old roof with a new one of corrugated iron.
- Installing new windows and doors.
- (if possible) removal of all or part of central wall.
- Construction and installation of an inoculation chamber and an incubation (dew) chamber, as detailed in Proposal 2.

EIAR Ambo Ethiopia 15-18 June 2008

Laboratory infrastructure

Lab facilities currently designated to the pathology group are small and consideration should be given to more space. The laboratory requires:

- minus 80°C refrigerator
- microwave oven (for drying silca gel)
- desiccators to allow drying of rust spores
- Eppendorf tubes (cryovials) with screw lids

Greenhouse infrastructure

There are two large greenhouses, one of which is currently in use and has limited (almost non-functional) air conditioning (Appendices II-2A, II-2B, II-2C, II-2D). The second greenhouse (Appendix II-1) is non-functional and would require significant funding to recommission. Given the level of funding available, upgrading the first greenhouse is the only realistic option at this point.

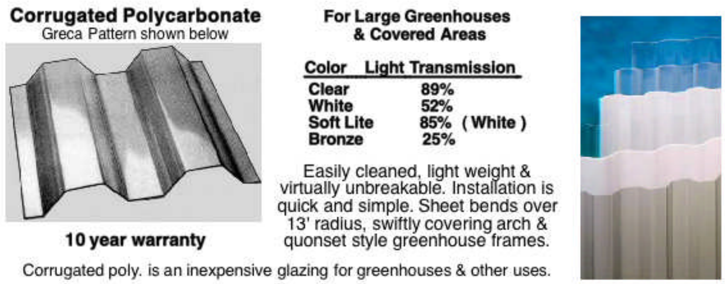
A floor plan for the first ("Main") greenhouse is given in Appendix II-2C. It consists of a central passage with several independent rooms on both sides. The entire complex needs extensive upgrading in terms of new glass or polycarbonate. Some partitions are solid walls and these may be replaced by polycarbonate to improve light. At present there is no functional air conditioning system and at least the four rooms managed by the Plant Pathology section should be upgraded.

The most effective approach to upgrading greenhouse facilities at Ambo will be to refurbish several compartments of the "Main greenhouse", to accommodate the raising of healthy seedlings, inoculation, incubation (dew treatment), and then post-inoculation incubation.

<u>Proposal</u>

General layout Ideally all stem rust work should be consolidated at one end of the greenhouse (compartments 1, 2, 3a, 6 and 7, Appendix II-2C). This could be achieved by exchanging two rooms with the entomologists and will provide two north-facing and two south-facing rooms on opposite sides of the central passage. These rooms are adjacent to three smaller rooms (compartments 1a, 1b and 1c, , Appendix II-2C) which have to be remodelled as inoculation and incubation facilities.

Specific requirements Reglazing of entire greenhouse roof and partitions. It is advisable to use corrugated polycarbonate sheets as they are light, easy to handle and very strong. Depending on the colour selected, light transmission is in the range of 25%-89%. Examples are shown below.



Alternatively, greenhouse specific polycarbonate sheeting could be used (e.g. Lexan Thermoclear; http://www.ampelite.com.au/images/LEXAN%20Thermoclear%20Plus%20Flyer.pdf). While this product has superior strength and insulation properties compared to corrugated polycarbonate, it is more expensive.

The four rooms allocated for rust research (compartments 2, 3a, 6 and 7) need air conditioning in the 15-25°C range. It is envisaged that each room will have a separate air conditioner. The cooling capacity should be calculated based on the size of the room as well as environmental conditions of the region. As a guideline, a unit responsible for conditioning temperature in a 6.25 m² greenhouse cubicle at UFS, South Africa, has a capacity of 50400/55600 btu/h (see picture below). The air conditioners should also have heating elements and an electronic control system which can be set to the desired day/night cycles. In many cases such control systems are custom built. Consideration should also be given to a concrete slab or other structure to hold the conditioning unit in place. Further requirements are ducting between the unit and the greenhouse and a louvred inlet which blows vertically into the room. The extraction duct is also fitted with a filter which needs regular cleaning. All four compartments should be fitted with retractable internal heat blankets.

Example of air conditioning unit, ducting and inlet in greenhouse cubicle

Each greenhouse room needs electricity (for operating spore collecting devices) running water and an outlet to drain extra water from the floor.

The inoculation room (Appendices II-2C and II2D, compartment 1c) needs a new door (to close off dissemination of spores) and general facilities for efficient inoculation,e.g. inoculation booth (an example is shown below), running water, electricity and bench space. Since water pressure has been indicated as a problem area at Ambo, we suggest that an elevated reservoir is erected next to the greenhouse.

The existing incubation room (Appendix II-2D) should be remodelled to allow the installation of ultrasonic humidifiers connected to time controllers. Based on experience as many as three humidifiers may be necessary for the existing space. It is preferable to use distilled water in these devices. Shelving on wheels (three units should be constructed and galvanised to increase capacity in this room.

The minus 80° freezer could be placed in the inoculation room if space and back-up electricity are sufficient. Otherwise this could be situated in the plant pathology laboratory.

All other equipment needed for rust research and listed above, should be obtained, manufactured or improvised. As race analyses will be conducted here, special attention should be given to isolation facilities for a number of different isolates and for minimizing contamination of differential sets and/or seedlings for increase field collections.

A covered facility for cleaning of pots and storage of potting mix can easily be constructed at the eastern entrance to the greenhouse.

EIAR Debre Zeit Ethiopia 17 June 2008

Although Debre Zeit has not been earmarked to do the official Ethiopian stem rust race analysis, our understanding was that they need basic infrastructure for routine screening and inoculum increases. This requires all the basic facilities for rust research, although less emphasis will be placed on handling and isolating different cultures.

Proposal

Greenhouse Three facilities are available for upgrading (Appendices III-1A, III-2A, III-2B). According to Dr Ayele their preference would be to upgrade the "Partially completed greenhouse" unit closest to the lab (Appendices III-2A, III-2B). To be transformed to a functional greenhouse, this facility will need extensive renovation, e.g. a new roof structure (presently rudimentary eucalyptus rafters; Appendix III-2A), roof and walls (brick wall to be lowered for more light penetration) of polycarbonate, internal construction within compartment 3 of four rooms, air conditioning for each room, installing retractable internal heat blankets in each, cement floors, running water, electricity and potting facility. The adjoining two smaller rooms (compartments 1 and 2, Appendix III-2A), can be cleaned and refurbished to house the inoculation and incubation equipment. These items have to be custom built.

Although not specified, a minus 80° freezer is required for preservation of cultures. Other equipment required includes:

- minus 80°C refrigerator
- microwave oven (for drying silca gel)
- desiccators to allow drying of rust spores
- Eppendorf tubes (cryovials) with screw lids
- ultra low volume applicator for field inoculations

No back-up generator was specified, but is important for the sustainability of a rust research facility.

The installation of an overhead misting system in the lath house (Appendix III-3), to promote rust epidemics in nurseries, is supported. This could be constructed of inexpensive 20 mm plastic piping, elbows, connectors and microjets. It can be a manually operated system.

EIAR Kulumsa Ethiopia 18 June 2008

Since Kulumsa is an important station for bread wheat research in Ethiopia they also have the need to do rust work. We were impressed with the progress already made in establishing infection with stem rust and stripe rust using what is currently available, and feel that this facility will be the easiest of the three we inspected (Ambo, Debre Zeit and Kulumsa) to upgrade. The existing greenhouse (Appendix IV-1A, IV-1B) is structurally in good condition but needs more effective air conditioning. Some glass panes need replacement.

We suggest the addition of suitable air conditioners and control systems that will maintain temperatures between 15 and 25°C and the installation of retractable internal heat blankets. The pathology lab (Appendix IV-2) is small but suitable for basic rust work and already has equipment such as a waterbath, a pressure pump, and an operational (but currently unused) growth cabinet. The minus 80° freezer is not functional and from experience known to require special expertise to repair. The acquisition of a new freezer is recommended. The two smaller rooms (compartments 1 and 2, Appendix IV-1B) at the greenhouse entrance could easily be refurbished as inoculation and incubation rooms.

The installation of air conditioners in the seed rooms (Appendix IV-3) is supported. Similar to Debre Zeit the installation of an inexpensive overhead misting system in the lath house (IV-4) is supported. Other dedicated rust equipment required includes:

- microwave oven (for drying silca gel)
- desiccators to allow drying of rust spores
- Eppendorf tubes (cryovials) with screw lids
- Ultra low volume applicator for field rust inoculations

A back up power generator is also needed (they currently do not have one).

Concluding remarks

The three Ethiopian centres should join forces in upgrading the greenhouses. It may be worthwhile to appoint architects/engineers to oversee the electrical, air conditioning and structural installations/upgrading. Acquisition of items such as inoculation booths, dew chambers, inoculating equipment, freezers, etc. should preferably be done jointly. For back-up generators there are many technical things to consider such as the KVA requirements of the facility, location, existing cabling and wiring, automated response, noise, cooling, ventilation, storage and availability of diesel, etc.

In general special attention has to be given to a maintenance plan and therefore selection of reputable dealers is extremely important.

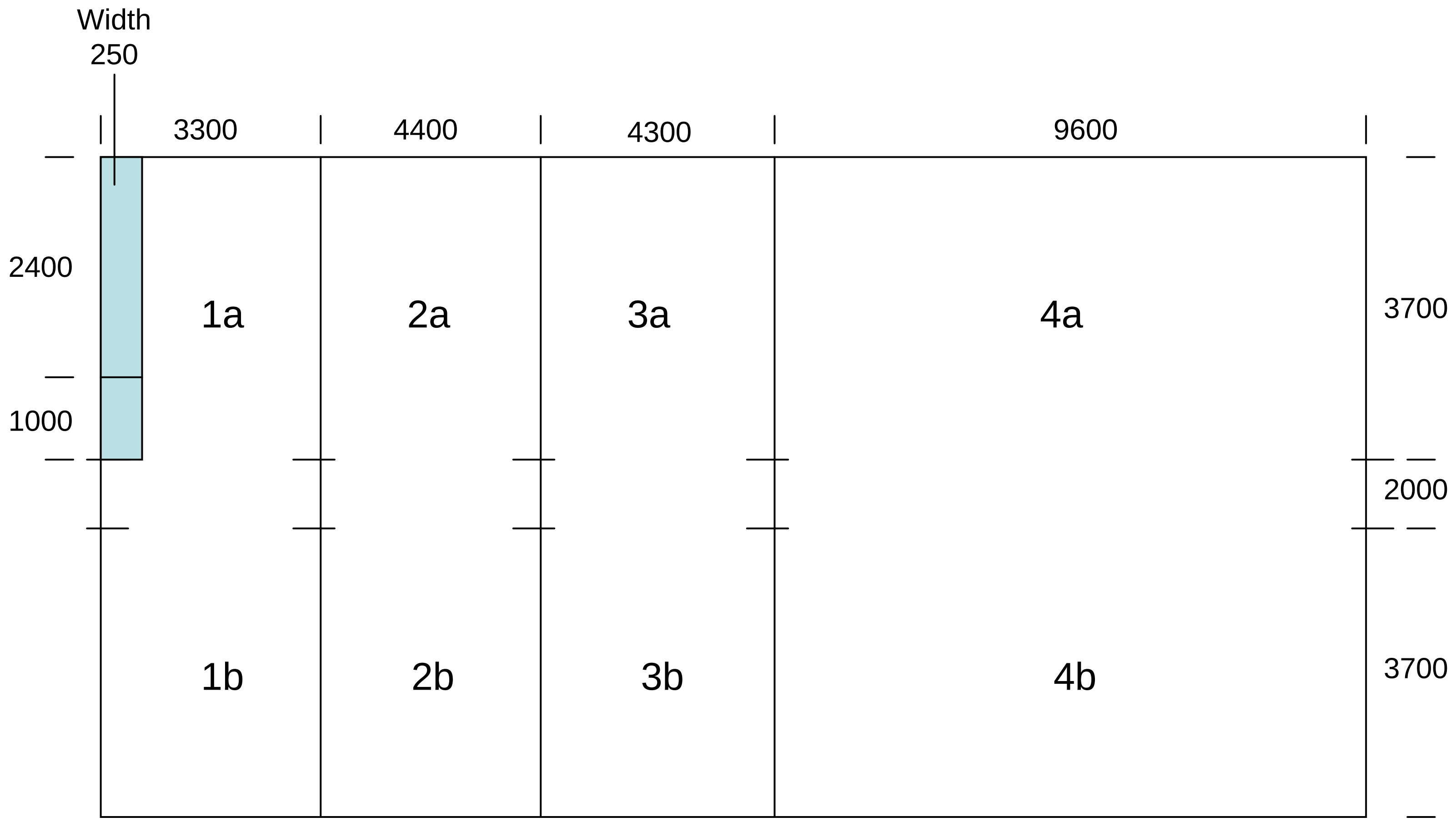
Appendix I-1A. KARI Njoro, “Main greenhouse”

“Main greenhouse”

Compartment 1a, showing sink

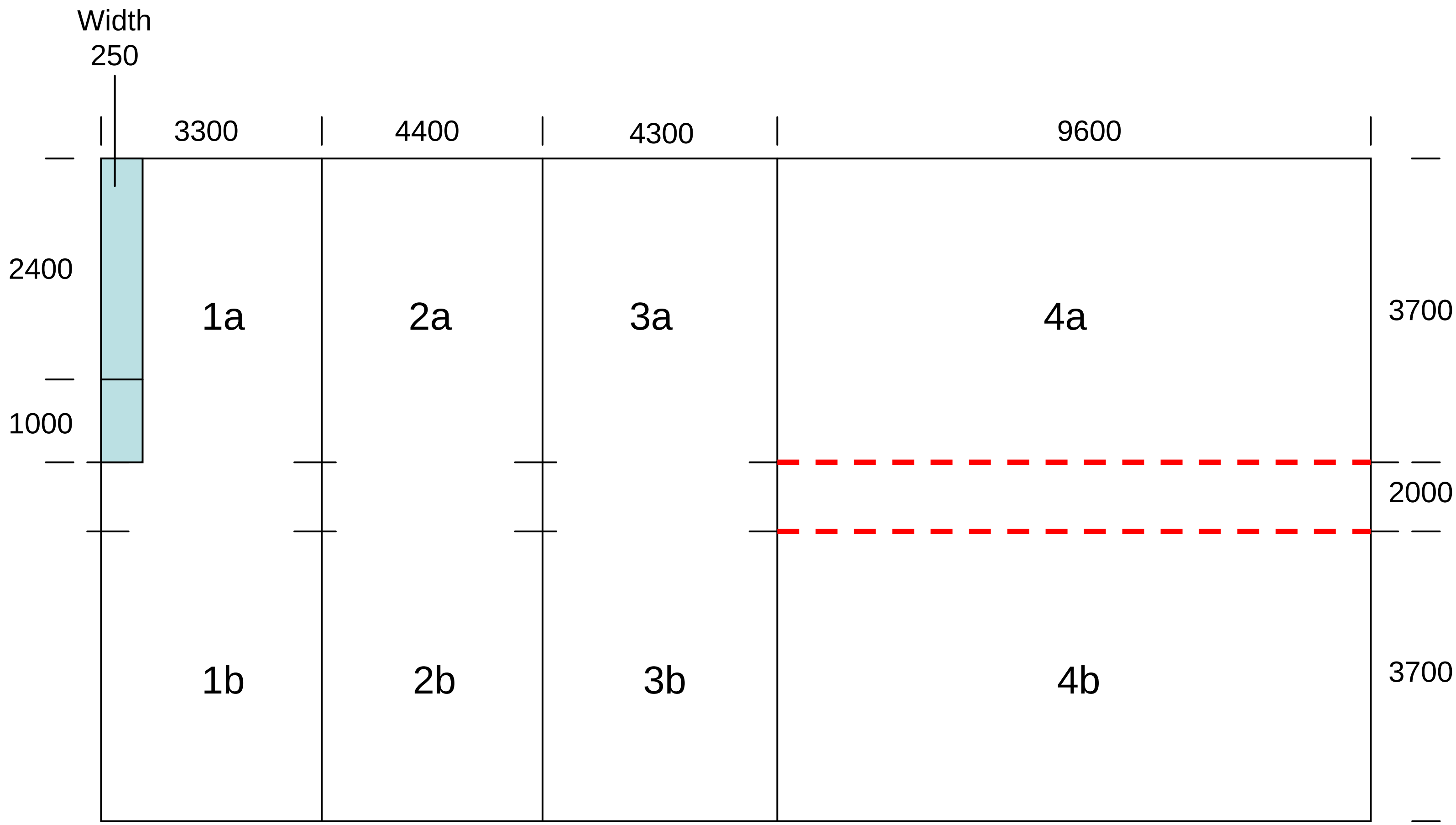
Compartment 4a, showing duct for air circulation

Appendix I-1B. KARI Njoro, “Main greenhouse” floor plan



“Main greenhouse”
Four compartments, central access, bench and sink (shown in blue)
(measurements are approximations)

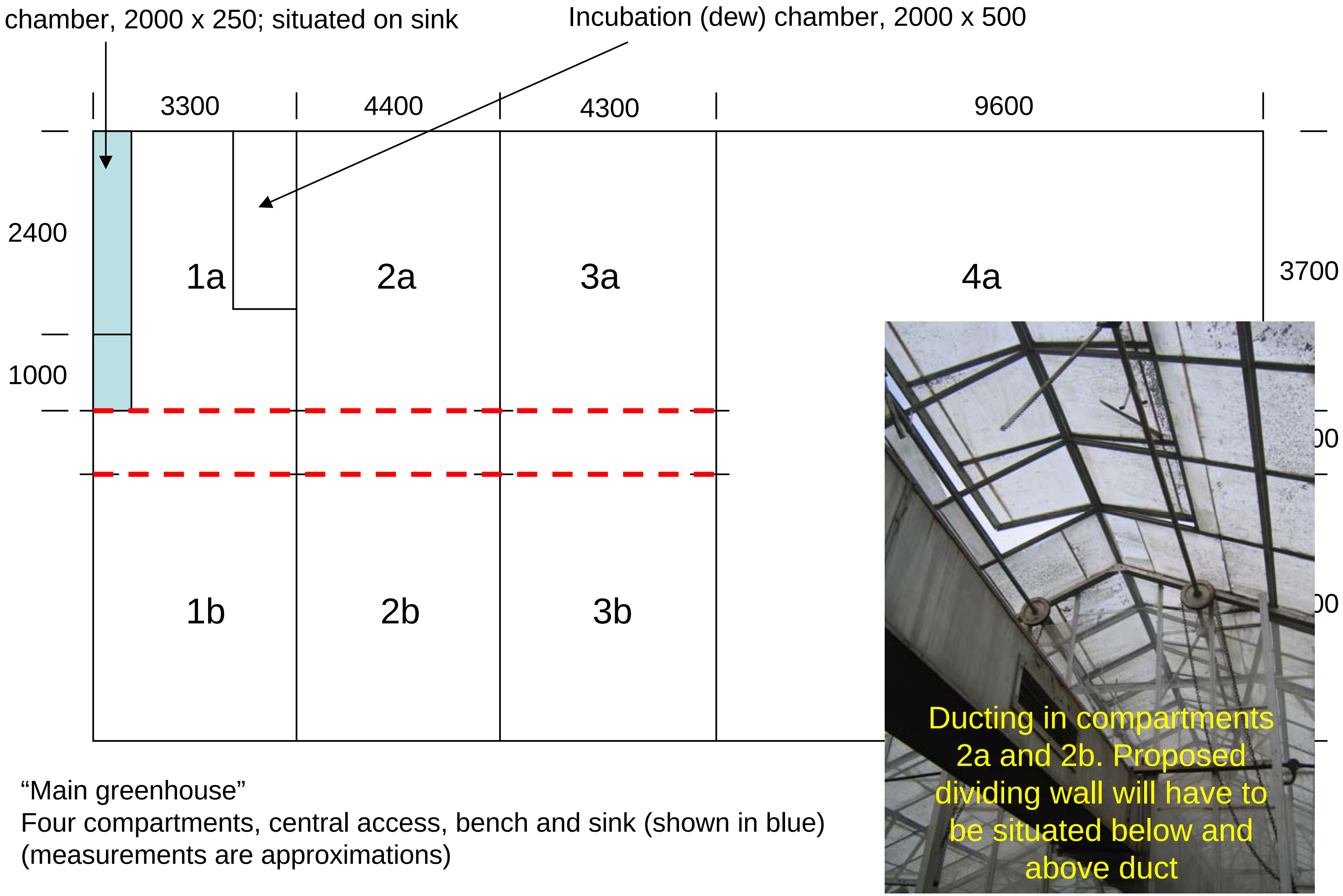
Appendix I-1C. KARI Njoro, "Main greenhouse" floor plan, Proposal 1



"Main greenhouse"
Four compartments, central access, bench and sink (shown in blue)
(measurements are approximations)

Proposed new walls shown in red dash

Appendix I-1D. KARI Njoro, “Main greenhouse” floor plan, Proposal 2

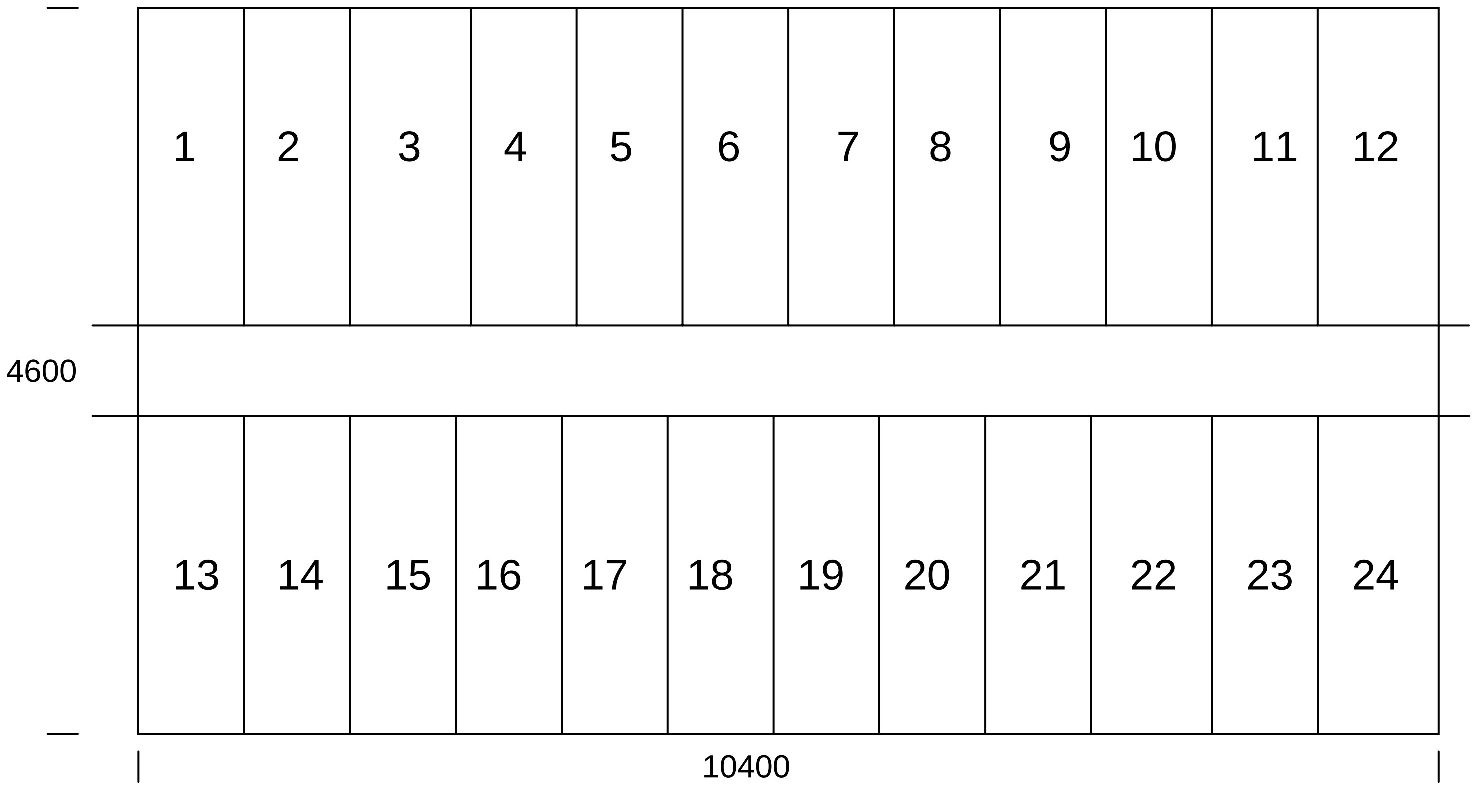


“Main greenhouse”
Four compartments, central access, bench and sink (shown in blue)
(measurements are approximations)

Proposed new walls shown in red dash

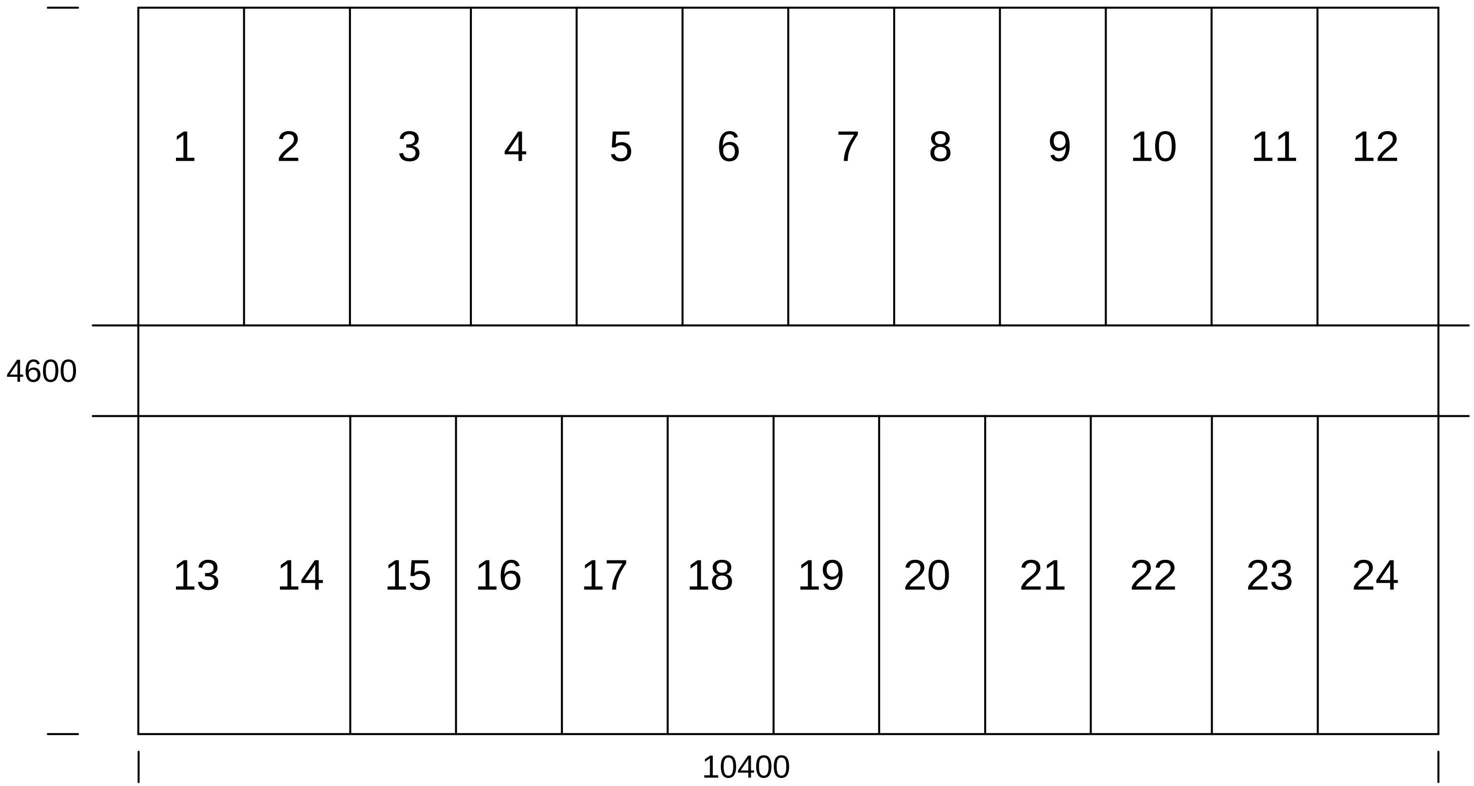
Appendix I-2A. KARI Njoro, “Rust isolation facility”

Appendix I-2B. KARI Njoro, “Rust isolation facility” floor plan



Central corridor with doorway access at each end.
24 individual compartments each with venting system
(measurements are approximations)

Appendix I-2C. KARI Njoro, “Rust isolation facility” Proposal 1 modifications



Central corridor with doorway access at each end.
24 individual compartments each with venting system
(measurements are approximations)

Removal of wall between compartments 13 and 14 to make an inoculation room.

Appendix I-3A KARI Njoro, “Former stripe rust facility” (two buildings on left) and adjacent “Small Greenhouses” (right)

Appendix I-3B KARI Njoro, “Former stripe rust facility” floor plan

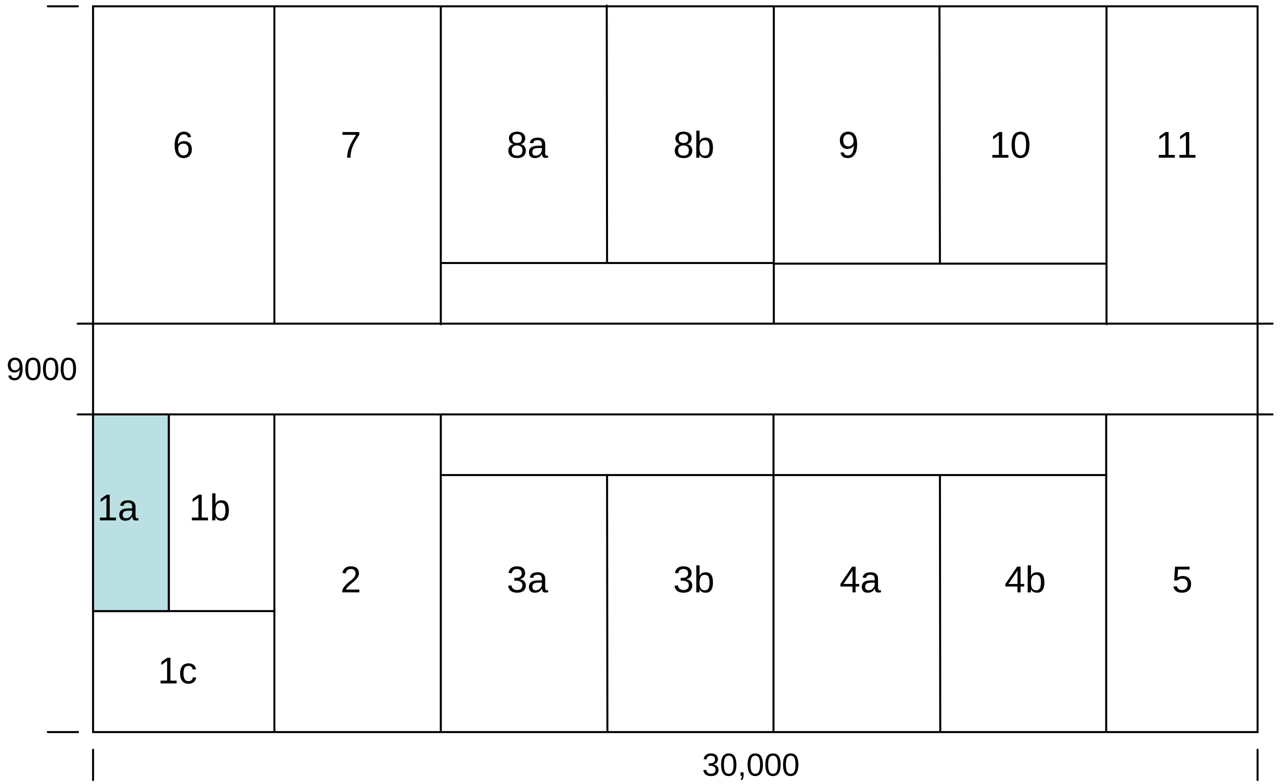
Doorway access and corridors down each side, middle (longitudinal) wall constructed from brick, central wall is a temporary structure.

Appendix II-1. EIAR Ambo, “Old greenhouse”

Appendix II-2A. EIAR Ambo, “Main greenhouse”

Appendix II-2B. EIAR Ambo, “Main greenhouse”

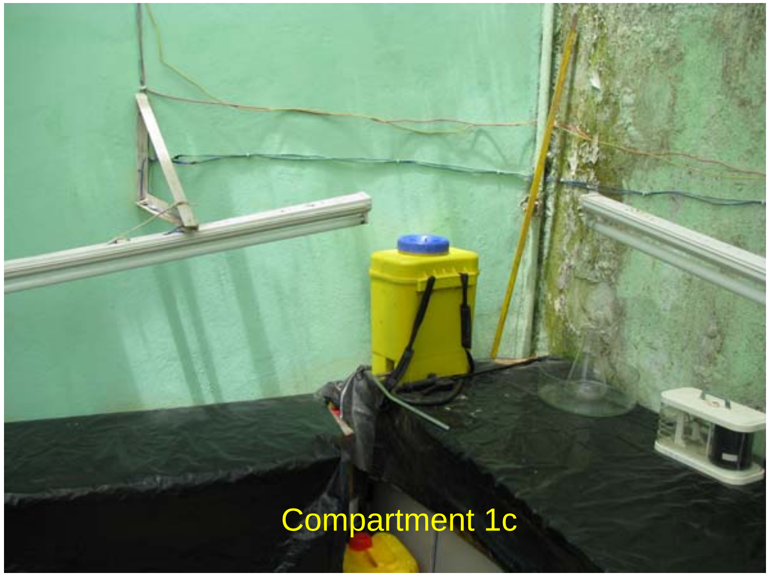
Appendix II-2C. EIAR Ambo, “Main greenhouse”, floor plan



Central corridor with doorway access at each end.
Existing dew chamber (shaded blue)
(measurements are approximations)

Appendix II-2D. EIAR Ambo, “Main greenhouse”

Existing incubation room (compartment 1a)

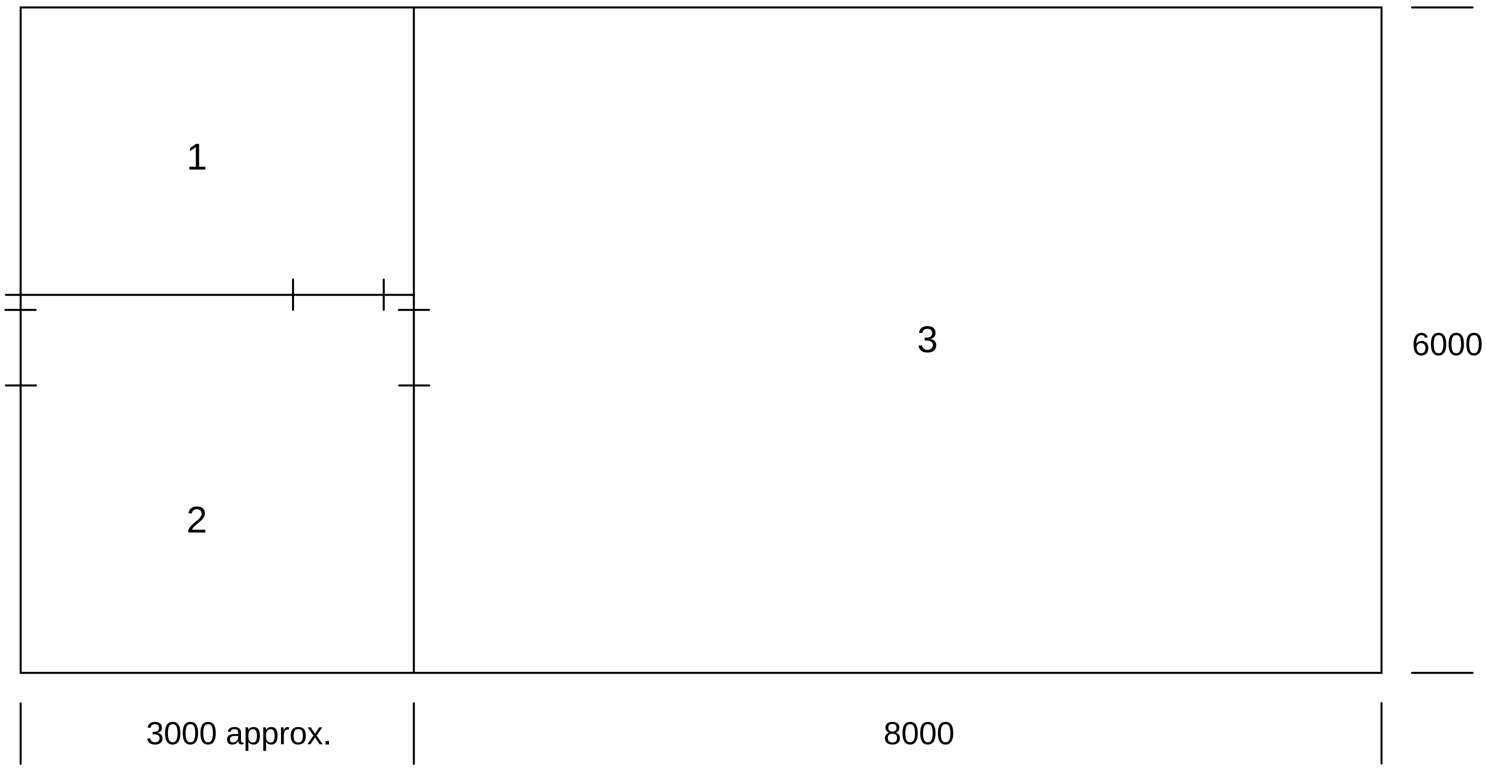


Compartment 1c

Appendix III-1A. EIAR Debre Zeit, Greenhouses “1” and “2”

Appendix III-2A. EIAR Debre Zeit, “Partially completed greenhouse”

Appendix III-2B. EIAR Debre Zeit, “Partially completed greenhouse”, floor plan

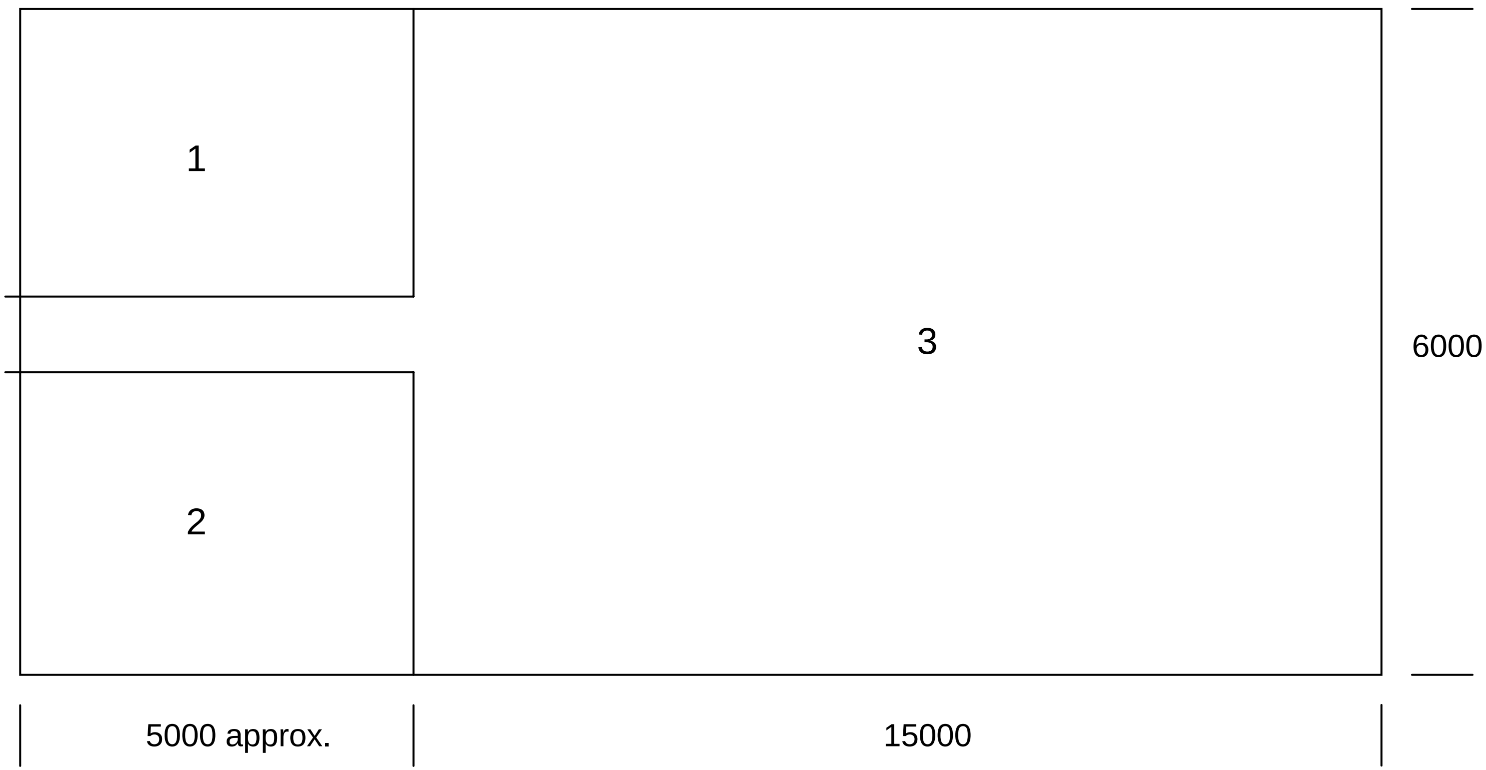


(measurements are approximations)

Appendix III-3. EIAR Debre Zeit, Lath-house

Appendix IV-1A. EIAR Kulumsa, “Greenhouse”

Appendix IV-1B. EIAR Kulumsa, “Greenhouse”, floor plan



(measurements are approximations)

Appendix IV-2. EIAR Kulumsa, Plant Pathology Laboratory

Operational growth cabinet (not in use)

Appendix IV-3. EIAR Kulumsa, Seed Stores

Appendix IV-4. EIAR Kulumsa, Lath house