Mushroom Cultivation in Mamre, Western Cape, South Africa

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I. Introduction

The Agricultural Research Council (ARC) of South Africa has coordinated the construction of low-tech shipping container-based oyster mushroom grow houses at a rural development site in Mamre, Western Cape, South Africa. The purpose of this project is to provide a means of revenue generation for underemployed citizens of Mamre. Oyster mushroom cultivation can be accomplished with relatively little technological input on non-electrified sites and is a plausible means of wealth generation for rural communities in South Africa.

The ARC's rural development site in Mamre houses low-tech mushroom growing houses fashioned from modified refrigerated shipping containers (Photo 1). The site does not have an

Photo 1: Left) Refrigerated shipping containers modified for use as mushroom grow houses in Mamre. Right) Inside view of grow houses showing shelving used to hold mushroom fruiting bags.

electricity source, making temperature, lighting, humidity, and cleanliness controls difficult. These deficiencies make it an ideal site for the development of cultivation techniques for communities lacking municipal services, a condition common among many rural communities in

South Africa. Temperature and sanitation control equipment are also prime targets for vandalism and theft, so it is important that small growers have the means to operate without them.

Our work training farm workers in Mamre has shown that it is possible to cultivate oyster mushrooms under less than optimal growth conditions. The techniques developed for farm workers at the Mamre site are readily transferrable to other rural development projects in South Africa. This document is intended to serve as a guide to oyster spawn and mushroom production for small-scale mushroom farmers and project coordinators.

II. Standard procedures for fungal culture maintenance and spawn production

Spawn is one of the largest input costs for mushroom farmers, and a low-cost source of high-quality spawn greatly improves the viability of small-scale operations. The ARC Nietvoorbij campus hosts several microbiology laboratories and has ample laboratory space and equipment to produce mushroom spawn for rural development projects. In the long run, the ARC's lab space can be used to produce low-cost mushroom spawn for mushroom cultivation projects. The following are short protocols for the production of nutritive agar, mushroom spawn, and mushroom-growing substrate.

A. Agar medium for oyster mushrooms

Agar is used for the maintenance and propagation of fungal cultures. When making spawn, agar pieces covered with the oyster mushroom mycelium are used to inoculate sterilized grain. For long term storage cultures are grown on agar at room temperature until the medium is

fully covered, then stored at 4˚C for up to 6 months before transferring to fresh medium. The first step in producing spawn is to propagate oyster mushroom mycelium onto enough agar plates to inoculate the desired amount of spawn. Below is a simple recipe for agar medium using readily available ingredients.

Materials:
Agar
Nutrifics biscuits, Maltabella sorghum porridge, or other malt-containing cereal
Distilled water
Sterile, disposable plastic petri plates
Parafilm
70% ethanol in a spray bottle

Equipment:
500 ml Erlenmeyer flask
Stir bar
Hot plate with magnetic stirrer
Autoclave (or pressure cooker)
Laminar flow cabinet

Procedure for 250 ml of agar medium:

1) Weigh out 6.25 g of Nutrifics biscuit or maltabella sorghum porridge (carbon source) [a].
2) Weigh out 3.75 g of agar[b].
3) Measure 250 ml of water and place into 500 ml flask[a].
4) Add agar and carbon source to water.
5) Heat water on hot plate while stirring with magnetic stir bar.
6) Heat until the agar melts, when this occurs no agar particles should be visible as they are dissolved in the water. Make sure to watch the flask as it gets hot so that it does not boil over.
7) Cover flask with Aluminum foil so it is protected from exposure to air.

8) Autoclave the flask containing the melted agar for 15 minutes, allow slow venting and cooling on the liquid cycle. A pressure cooker can also be used to bring the media to a high enough temperature.
9) Clean a work surface in a laminar flow cabinet with 70% ethanol spray.
10) Take hot agar out of the autoclave and place it in the laminar flow hood with the fan on to cool.
11) Allow to cool for about 10 minutes (longer if you are making a larger volume).
12) Open a packet of sterile petri plates in the laminar flow hood making sure to keep the lids on to avoid contamination.
13) Pour enough hot media into each plate to cover the bottom. In the end the agar should be about 0.5-0.75 cm thick on the bottom of the plate. 250 ml of agar should be enough to pour 10 plates (Photo 2).
14) Leave each plate propped open slightly in the laminar flow hood until it solidifies, about 15 minutes (Photo 2). Then close the lids and stack the solidified plates and enclose in their original packing, taped shut and labelled with the media type and date.

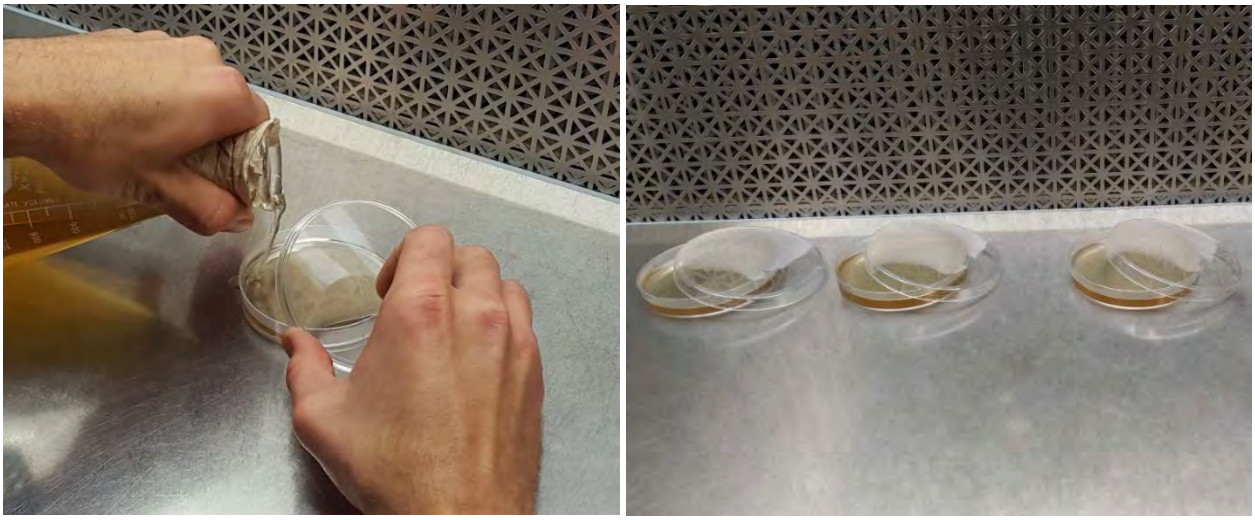

Photo 2: Left) Hot agar is poured into petri plates in a layer about 0.5-0.75 cm thick. Right) Hot agar petri plates are propped open and left to cool in the laminar flow cabinet to prevent condensation buildup.

15) Store plates at 4˚C, upside-down until they are needed.

Notes

a) This recipe is for a 2.5% (g/ml) carbon source medium. The amounts listed here can be scaled up for any volume of agar medium that needs to be made.
b) This recipe calls for 1.5% agar, scale up as needed. Agar can be obtained from health food stores. Alternatively, lab grade agar can be purchased and used just the same.

B. Mushroom spawn

Mushroom spawn is made by inoculating sterilized grains with a strain of mushroom forming fungus using a pure culture of the fungus grown on agar plates. Typically, small to medium grains such as wheat, rye, or sorghum are used. Larger grains such as maize are not

ideal because they result in fewer inoculation points in the substrate. Very small grains such as millet tend to stick together in clumps also limiting dispersal. Seed or feed grade grains are acceptable for spawn production as the mushroom spawn is not directly consumed by humans. Grains are readily available from local agricultural supply stores.

Timing of mushroom spawn production is very important in order to maintain a constant supply for mushroom growers. Once a date has been set for mushroom spawn inoculation, the finished product will take anywhere from 2-4 weeks to complete growth, depending on how much grain is inoculated and how much inoculum is used. Mushroom spawn can also be produced in bulk, grown to maturity, and stored at 4˚C for up to two months and distributed as needed to farm workers. To save labor, a two-month supply of mushroom spawn should be produced at a time and stored until needed. The fresh mushroom spawn should be inoculated before half of the two-month supply is depleted so that there is no lapse in supply.

Bulk mushroom spawn is typically made in autoclavable polypropylene bags with respirator patches because of their larger volume and malleability. These can be obtained from a local mushroom grower (several exist in the Western Cape province) or from international suppliers. Mushroom spawn can also be produced in glass jars with a small hole punched in the lid, plugged with cotton. Jars are reusable and do not require a bag sealer, but are limited in the amount of spawn that can fit in them (typically 2 liter jars are the largest manageable size).

Mushroom spawn production requires long soaking and sterilization steps and the procedure spans roughly 18-20 hours. It is recommended that the procedure be broken into two days with the grain soaking step done overnight. The following is a recommended protocol for the production of mushroom spawn on wheat grain.

Materials:
Whole wheat grain, seed grade or food grade
Newspaper
Water
Autoclavable polypropylene mushroom-growing bags
Actively growing edible mushroom cultures
70% ethanol in atomizer bottle
95% ethanol in small jar

Equipment:
Plastic 20 liter buckets
Jars with punctured, cotton-sealed lids
Impulse plastic sealer (only if using mushroom growing bags)
Scalpel
Alcohol lamp containing 95% ethanol

Procedure for mushroom spawn production:

Day 1

1) Place a desired amount of wheat grain in a bucket(s) and cover it with room temperature water[a].
2) Allow the wheat grain to soak for 8-10 hours at room temperature

Day 2

1) Spread out newspaper on a clean, flat table. It is best to double layer the newspaper.
2) Drain the excess water from the wheat grains. The grains should be noticeably larger than they were before soaking, but not completely cracked open at the ends.
3) Spread the wet grains on the newspapers. Make sure that the grains are spread in a thin layer so that each grain is in contact with the newspaper, not stacked on top of another grain.

4) Allow the grains to drip dry for 1 hour. The grains should be moist but not with excessive amounts of free moisture on the surface. Their final moisture content should be about 45%.
5) Pack the grains into 2-liter glass jars or autoclavable polypropylene bags with respirator patches[b].
6) Autoclave the grains for 90 minutes at 120˚C, 15 psi on a slow-venting liquid cycle.
7) Place the hot jars in a laminar flow cabinet cleaned with 70% ethanol to cool.
8) Allow the grains to cool to room temperature, about 2 hours.
9) Inoculate the grains with the appropriate mushroom cultures growing on agar. This is done by cutting a fully colonized agar culture into 1 cm^2 pieces with a sterile scalpel and transferring 3-4 agar pieces per 2-liter jar into the sterilized grain using the sterilized scalpel (Photo 3).[c]
10) After each transfer the scalpel must be re-sterilized in 95% ethanol. All culture manipulations must be done in sterile conditions within a laminar flow cabinet using proper sterile technique (see instructional on sterile technique below).

Photo 3: Left) Agar squares colonized with oyster mushroom mycelia are aseptically added to sterilized grain. Right) The grains are covered with white mycelia of the oyster mushrooms. Colonization is complete once all grains are covered.

Incubation

1) Close the jars and remove from the laminar flow cabinet. Incubate in the dark at 20-25˚C until the grain is fully colonized. This will take about 3-4 weeks, depending on the temperature and the amount of agar added to the jars.
2) The colonization of the grain can be monitored visually, and the grains will become covered with a white mold as the fungus grows over the grain. The grain should be completely colonized before it is used (Photo 3).
3) Monitor the jars regularly for contamination. Contamination will typically appear as a green or grey mold that inhibits the spread of the white mold growing from the agar patches. Contaminants grow much more quickly than mushroom mycelium and can appear in spawn jars overnight.

4) Remove any jars that contain contamination and discard the grain.
5) Once fully colonized with mushroom mycelium the spawn can be used immediately to inoculate mushroom substrate, or stored at 4˚C for up to 2 months.

Notes

a) The amount of grain soaked is dependent on the amount of spawn that needs to be made. An estimate of the amount of spawn needed for a particular growing operation should be determined prior to inoculation. Several types of cereal grains can be used including wheat, rye, oats, barely, and sorghum.
b) Polypropylene mushroom growing bags can hold a greater amount of mushroom spawn and are often more desirable for spawn production. However, because they hold a greater amount of mushroom spawn, they benefit from larger inoculum sizes which can be attained by using mature grain spawn rather than agar pieces. Polypropylene bags also require an impulse plastic sealer in order to seal the bags above the respirator patch after inoculation.
c) Make sure that at least one agar piece has its mycelia-side in direct contact with the sterile grain. More agar pieces will result in faster colonization rates.

C. Best practices for fungal tissue culture

The manipulation of edible mushroom cultures requires that they be kept free of any airborne or skin-borne contaminants. Generally, edible mushroom cultures are slow-growing and are easily out-competed by fast-growing molds. This is problematic for small scale farmers who wish to maintain fungal cultures and produce mushroom spawn in the absence of sterile

conditions. Sterile conditions made possible by a laminar flow cabinet are essential for reproducible mushroom culture maintenance and spawn production. The following are general guidelines that should be followed during fungal culture manipulation to avoid contamination.

Materials
Petri plates and fungal cultures
70% ethanol in an atomizer bottle
95% ethanol in a jar
Parafilm

Equipment
Autoclave or pressure cooker
Laminar flow cabinet
Scalpel or other manipulation tools
Alcohol lamp with 95% ethanol

Procedures

1) All culture manipulations where fungal cultures are directly exposed to air should be done in a laminar flow cabinet. If this equipment is not available manipulations should be done in a clean, enclosed space protected from outside air currents.
2) All media on which fungal cultures are grown must be sterilized prior to use. Sterilization must be done in an autoclave or a pressure cooker at 120˚C.
3) All surfaces within the laminar flow cabinet or other workspace should be wiped down with 70% ethanol or other disinfectant solution prior to culture manipulation.
4) Fungal cultures should only be directly touched with rust-free metal tools. All tools that come in contact with fungal cultures should be sterilized in 95% ethanol and then placed through a flame to burn of excess ethanol.
5) Scalpels or other manipulation tools should be set into a jar of 95% ethanol between uses in order to re-sterilize. Before touching cultures again, the alcohol on tools must be

burned off to prevent it from killing off cultures. Tools should be allowed to cool briefly after being flamed.

6) Fungal cultures in petri plates should only be opened when they are being directly manipulated and should be closed when not in use.
7) Plates should be oriented such that the user's hands do not pass over the open fungal cultures when transferring the cultures to another container.
8) All petri plates need to be sealed with parafilm around the edges of the cultures as soon as they are no longer in use to prevent moisture loss and contamination.

III. Oyster mushroom cultivation

Oyster mushrooms can be grown on a variety of plant-based agricultural wastes. The substrate (most commonly wheat straw) must be sterilized or pasteurized prior to adding mushroom spawn. Commercial scale growers typically pasteurize mushroom substrate prior to inoculation because it is less energy intensive and is less susceptible to contamination after pasteurization than if it was sterilized. Pasteurization at the ARC rural development site and other non-electrified sites is accomplished by heating mushroom substrates to 70-75˚C for 1.5 hours in a hot water bath, draining, then cooling in open air. This technique requires minimal equipment and can be fueled by wood fires.

Oyster mushroom (*Pleurotus sp.*) can be cultivated under a variety of growth conditions and are adaptable for growth on a number of different pasteurized plant-based substrates, making them ideal for use in low-tech growing operations. Oyster mushroom are also a high value specialty mushroom in South African markets and can provide a large return on investment for farm workers with access to wealthy markets such as Cape Town. The following is a general

protocol for the preparation of oyster mushroom growing substrate and its inoculation. Also included are general guidelines for fruiting oyster mushrooms under poorly controlled conditions endemic to non-electrified mushroom farms.

A. Wheat straw pasteurization and inoculation

Materials
Wheat straw[a]
Water-enough for about 2/3 of a 200 liter drum
Polyethylene bags or tubing (at least 50 µm thickness)[b]
Rubber bands
4 cm diameter PVC piping cut into segments 4-6 cm long
Rubber gloves
70% ethanol in a spray bottle
Towels
Bleach
Grain mushroom spawn
Fuel wood
Cotton wool

Equipment
200-liter metal drum
Cinder blocks or another means of elevating a 200-liter drum above a fire
Plastic folding tables
Cleaned pitchfork or turning fork
Bricks or other things that can be used as weights

Procedure

1) Arrange cinder blocks or other support so that the 200-liter drum sits over an open space where a fire can be made (Photo 4).
2) Place the drum on top of the fireplace and fill it about 2/3 full with water.
3) Start a fire underneath the drum to heat the water.
4) Heat water to nearly boiling.

5) After water is hot, add enough wheat straw to fill in the drum. The wheat straw should be tightly packed in to the drum and completely submerged in hot water. The straw should be packed in with a clean garden fork or another tool (Photo 4).[c]
6) Place clean bricks on top of the wheat straw in order to hold it under the hot water.
7) Maintain the fire after wheat straw is packed into the drum as the temperature of the water will decrease after adding the wheat straw.
8) Take the temperature of the water at the top of the drum to make sure that it stays at or above 70˚C during the duration of the pasteurization. If a thermometer is not available, the fire should simply be maintained so that the water is kept near, but not at a boil.[d]
9) Pasteurize the straw for 1.5 hours.
10) Clean plastic folding tables with a diluted bleach solution to sanitize the surface.
11) Remove the straw from the hot water and place on cleaned folding tables (Photo 4).
12) Allow the straw to cool to ambient temperatures.
13) Pack straw into clean polyethylene plastic bags or polyethylene tubing modified to hold mushroom substrate.[b] Make sure the straw is packed tightly into bags to minimize airspaces.
14) When packing bags layer in mushroom spawn evenly throughout the bag. Each bag should contain about 2 kg of wet substrate. The total amount of spawn added to each bag should be about 4-5% of the total mass of the substrate (80-100 g per 2 kg bag), with higher rates leading to faster colonization.[e]
15) Close bags and allow to sit overnight. After sitting free water will have pooled at the bottom of the polyethylene bag. If using a bag with only one opening, a hole will need to

be cut in the bottom of the bag to drain free water. After draining the water, seal the hole again by taping the bag over itself.[f]

16) If using plastic tubing with a PVC pipe opening, allow the substrate to drain through the PVC opening overnight before stuffing the opening with cotton wool.

17) After draining, allow the bags to incubate at 20-25˚C until they are completely covered in

Photo 4: Left) Wheat straw is packed into a drum of hot water for pasteurization. Top right) Wheat straw is taken out of the hot water bath to drain and cool. Bottom right) Metal drums set on top of makeshift brick stands over a wood fire. The drums are filled with water and straw and the straw is held under with bricks during the pasteurization.

white mycelium.

Notes

a) Ideally wheat straw should be chopped before pasteurizing. If a shredder is not available then whole wheat straw can be used, but it is better to have smaller pieces of straw for mushroom cultivation.
b) Polyethylene bags should not be too large as they should only hold about 2 kg of wet substrate. Larger bags take longer to colonize increasing the risk of contamination and are also less efficient and result in substrate waste. Polyethylene tubing (Poly tubing) is a very versatile and economical option for making mushroom growing bags, but it must be modified to hold mushroom substrate.
    a. Protocol for converting plastic tubing into mushroom bags: Cut plastic tubing to appropriate length to hold about 2 kg of wet mushroom substrate plus extra for tying off ends, about 45 cm long for 30 cm wide tubing. Twist and tie one end of the tubing closed using a rubber band. Pack pasteurized straw into the open end of the tube and layer in spawn. Take a PVC piping piece and draw the open end of the bag through it and fold the edges of the plastic down over the tubing, holding it in place with another rubber band wrapped around the PVC pipe. Once the wet substrate is drained of free water from the PVC pipe opening, the opening is stuffed with cotton wool to prevent contaminants from entering while still allowing gas exchange.

c) Use caution when packing the drum with straw, if the water is too hot it may boil over once the straw is added. Do not over pack the drum, it is important that all of the straw remain in contact with the hot water.

d) Maintaining the straw at boiling for too long will sterilize the straw and make contamination inevitable. If the water is at a rolling boil, let the fire die down and let the water cool a bit. Ideally a thermometer will be available to check temperatures, but if not the water can just be kept just below boiling with a small fire or coals.

e) In practice farm workers will not have access to scales to weigh each bag and spawn additions will have to be done by eye. Farm workers should be given scoops that hold approximately the correct mass of spawn for inoculating a 2 kg bag so they can easily measure mushroom spawn.

f) For bags without openings, holes may need to be cut to provide aeration for the mycelium if colonization is slow. This greatly increases the risk of contamination and a much safer means of facilitating air exchange is by using bags made with plastic tubing that have a built-in opening for air exchange.

B. Fruiting, harvesting, and storing oyster mushrooms

After colonization of the mushroom substrate, environmental parameters must be altered to stimulate mushroom formation. In order to stimulate mushroom production there are four major environmental parameters that must be manipulated: temperature, humidity, light, and fresh air exchange. Oyster mushroom formation requires moderate temperatures (20-25˚C), some natural or artificial light, high humidity near 100%, and regular fresh air exchanges.

Environmental conditions are difficult to control without access to electricity and climate control equipment, but there are some ways farm workers can help optimize conditions in their

grow houses for mushroom production. The following are general guidelines for optimizing mushroom fruiting conditions in non-electrified grow houses.

Materials/Equipment
Rubber gloves
Hair net
Protective sanitary coat
Atomizer spray bottles
Pump sprayer
water
70% ethanol in atomizer bottle
Clean knife
Collection bin

Procedures

1) When in close contact to mushroom bags, farm workers should wear protective hair nets, gloves, and coat to minimize the risk of contamination. Farm workers should shower and put on clean clothes before working on a mushroom growing site.
2) Fresh air/oxygen: Once mushroom substrate is fully colonized it may be exposed to fresh air in the grow room. For plastic tubing remove the cotton wool in PVC piping and for normal polyethylene bags, cut 2 holes per bag in a position convenient for harvesting with a knife that has been sterilized with 70% ethanol. Exposure to fresh air raises the oxygen content of air in direct contact with the mushroom mycelium and stimulates fruiting. Once the bags are opened, grow room conditions must be moderated as described below to ensure proper mushroom development.
    a. If grow houses are completely filled with mushroom bags, fresh air exchange may become limiting and air will need to be circulated out of the grow houses. Typically, this is done with fans but in the absence of electricity this must be done manually by fanning air out of the grow house.

3) Temperature: Keep the cut bags at 20-25˚C. The grow houses at Mamre are well insulated and are ideal for maintaining moderate temperatures.
4) Light: non-electrified grow houses should have access to sunlight through windows on the rooftops. If these are not available, the grow rooms should be exposed to light from the outside multiple times per day. This can be done when the grow rooms are being humidified.
5) Humidity: It is vital to maintain high humidity (95%) in order to ensure maintain proper mushroom development. Without a humid environment, mushroom primordia (Photo 5) will not form or will dry out before reaching maturity. If adult mushrooms are exposed to low moisture environments, then they will rapidly dry out, lose quality, and become unsellable. It is especially important to maintain humidity in semi-arid climates such as the areas in the Western Cape near Cape Town.
    a. Farm workers should mist the air around mushroom grow bags at least three times daily, making sure not to soak developing mushrooms. Atomizer spray bottles or pumps are useful for getting moisture in the air. Farm workers will have to determine the optimal humidification regime for their conditions and it will likely vary depending on the time of year.

6) Mushrooms will develop over the course of about a week, depending on the temperature

Photo 5: Top left) Mushroom bag with cauliflower-like primordia emerging from a cut in the bag. Right) Mushroom bag with fully developed mushrooms emerging from holes cut in the bag. Bottom left) Harvested oyster mushrooms with slightly down rolled caps, an idea stage for picking.

of the grow rooms (Photo 5). It is critical that mushroom development be closely monitored during this time to ensure high quality mushroom production. Mushrooms can rapidly dry out or overdevelop, reducing their storability and quality.

7) Mushrooms are harvested once caps are fully expanded but still slightly down-curved at the edges.

8) Farm workers should harvest by gently plucking mushrooms, not by cutting them off with a knife. Cutting exposes damaged tissue to contaminants and increases the risk of contamination.
9) Once harvested mushrooms should be refrigerated immediately until they are ready to be taken to market. Quality decreases within hours of being picked if not refrigerated.
10) Oyster mushrooms can be dried if they cannot be sold fresh or if mushrooms are of lower quality. This is done by cutting the stems off of the mushrooms and setting them out to dry on a clean surface. Preferably this would be done in a dehydrator but on a non-electrified site, they must be sun-dried.
    a. Dried mushrooms can be powderized and mixed with salt and other spices for seasoning mix if they cannot be sold directly as dried mushrooms.

IV. Concluding remarks

Mushroom cultivation is a promising means of income generation for rural communities with access to wealthy markets. Low-tech grow houses such as modified refrigerated shipping containers are ideal for non-electrified sites common among underserved communities in South Africa. The Mamre site will continue to serve as a test-site for the development of techniques for oyster mushroom production at non-electrified sites.

The training exercises given to farm workers at Mamre have resulted in the successful production of a small batch of mushrooms with minimal guidance to farm workers. This is encouraging for the success of the project, although there are still some short-term barriers to success such as market access that are currently being resolved. Lessons learned by ARC

facilitators and farm workers at Mamre will continue to fine-tune this method of rural development in South Africa.