CUTICLE EXTRACTION PROCEDURE

FOR PINE

SAFETY: See associated COSSH form. Always wear PPE (lab coat, safety glasses and nitrile gloves). Inform safety officer of any spills.

5 % oxalic acid – burns, irritant
Methanol – toxic, highly flammable, irritant
Hexane – highly flammable, irritant
First aid – run with water for ~20 minutes, seek medical help for eye incidents.

CHEMICALS REQUIRED:

- MilliQ water
- Methanol
- 5 % oxalic acid
- Hexane

EQUIPMENT REQUIRED:

- 50 ml centrifuge tubes
- Water bath
- Dissecting microscope
- Balance / scales
- Eppendorf tubes
- Razors
- 2x fine point tweezers
- Watch glasses
- Al caps

NOTES:

The machine on which samples are run is very sensitive, chemicals should be as pure as possible (at least Aristar grade).
Equipment should be washed up without detergent, and rinsed with highest quality water (MilliQ) before use.

PROCEDURE:

1. Cut a 1 cm piece of pine needle with a razorblade. If possible separate the sun exposed and unexposed sides of the needle. Put the samples in 50 ml centrifuge tubes.

2. Add 5 % oxalic acid solution and leave for 1 hour in a boiling water bath. (AF said to cap samples)

3. With the use of tweezers carefully strip off the epidermal layer. It is important that you get a clean cuticle sample (you only want to know what's in the cuticle), probably need to do this under a microscope.

4. Rinse the epidermal layer 3 times in methanol. Place the material on a watch glass or microscope plate with cavities, hold with tweezers and rinse.

5. Rinse the epidermal layer 3 times in hexane (removes epidermal wax). As above.

6. Let them dry in air – can use Al caps or foil.

7. Weigh on a microbalance (don’t know how much mass will need to run per sample, you can have more than one piece of cuticle per sample but it shouldn’t be necessary, the method is very sensitive, and you shouldn't overload the machine).

8. Store the epidermal layer in small eppendorf tube. Samples should be stored dry in a dark place at room temperature.