METHODS: FREEZING MICROTOME

Brodo (1986, pers. comm.)

Mount specimen in mixture of gum arabic and phenol crystals.

Ice should be just hard enough to dent slightly with your fingernail.

Soak specimen (on slide, with label) in PhotoFlo, then put sections in watchglass of water.

Put 5 sections on each slide.

Lift sections from watchglass, put into cotton blue on slide, with brush.

Clean coverslip in water before putting on top of cotton blue; put coverslip on slowly with tweezers, with one end touching slide first, and try to avoid bubbles; use tiny drop of cotton blue, so it doesn't go past the coverslip. For permanent slid, wipe cotton blue with moist towlette, and seal twice with clear nail polish.

Hertel (1986, pers. comm.)

Ice should not be too cold (25oC is about right).

Soak specimen in alcohol first (prevents rupturing of tissues, but may dissolve substances).

Use thin, flexible tweezers, which will not squash the apothecia.

Attach disc side of apothecia to sloping surface of the ice.

Make ice flat by running a finger across it before the ice is completely frozen; add water in tiny amounts to avoid washing away the specimen.

Make at least 4 slides per specimen, at least 2 with cotton blue.

Put cotton blue on slide before putting specimen on. Leave sections in cotton blue for a while (at least several hours)_ before putting on the coverslip; this will stain the tissues better.

Adjust knife angle, experimenting until the knife neither shatters the ice nor glides over it, but actually slices it.

Section several apothecia of a specimen at a time, but remove each 34 sections, and put each set of 34 in a different drop on a slide.

Immediately after sectioning, before putting the coverslip on,and before the sections dry out, flatten and separate the sections and remove sand grains, using a needle under a dissecting scope.

From Roger Anderson

Put an insect pin inside a hypodermic needle and bend it to make a probe for manipulating ascocarps to be sectioned.