NECTARY COLLECTION AND RNA ISOLATION

Nectary collection

In our studies all plants used for nectary collection are grown under a 16 hour light/8 hour dark cycle, with nectary isolation occurring from 4-8 hours after dawn (h.a.d.). The rationale for this growth and collection scheme is that Arabidopsis flowers fully open by ~3 h.a.d., and nectar production in closely related *Brassica napus* peaks from mid-morning to mid-day (~4 to 8 h.a.d.). Thus we attempt to capture gene expression profiles in nectaries occurring during periods of active secretion.

All nectary tissues are separately dissected by hand from the flowers of primary inflorescences of ca. 30-35 day-old plants. Due to the small size of nectaries, dissections take place over several days from 4-8 hours after dawn (h.a.d.). Isolated nectaries are pooled in RNAlater™ solution (Ambion, Austin, TX) on ice, and stored at 4°C prior to RNA extraction. Up to two nectaries are collected per flower, with approximately 200-300 nectaries being processed as a single RNA sample, yielding approximately 500 ng of total RNA. Biological replicates are represented by nectaries pooled from different sets of plants. An example of nectary dissection can be viewed at: <http://mediamill.cla.umn.edu/mediamill/embedqt/165414>.

RNA isolation

RNA is extracted from floral nectariferous tissue by mechanical disruption with a microcentrifuge pestle, and using the RNAqueous®-Micro micro scale RNA isolation kit (Ambion, Austin, TX) with Plant RNA Isolation Aid (Ambion, Austin, TX). Denaturing agarose gel electrophoresis [68] and UV spectrophotometry are used to assess RNA quality for all samples.