Metabolic Engineering to Study the Regulation/Plasticity of, and to Modify Diterpene Metabolism in Trichome Gland Cells

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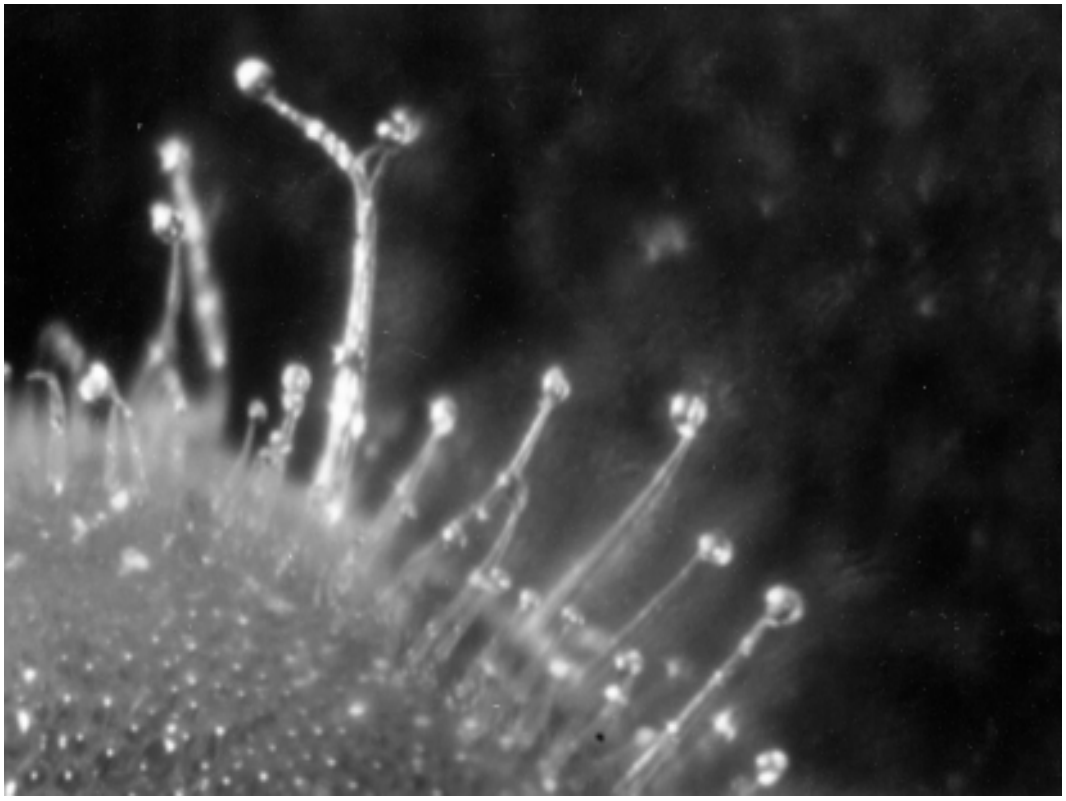
Project Objectives

1) To **investigate the regulation of carbon flow** in the biosynthesis of diterpenes produced in , and exudated by trichome glands.

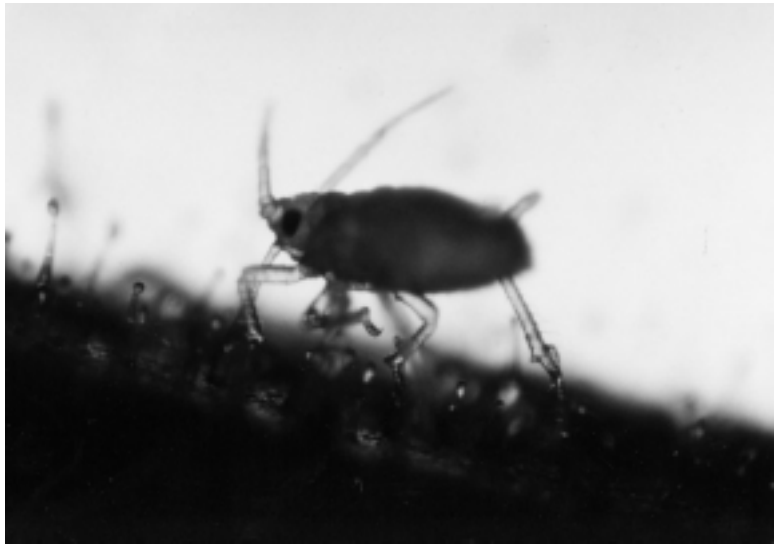
2) To study the **feasibility of modifying carbon flow** in glands to facilitate molecular farming.

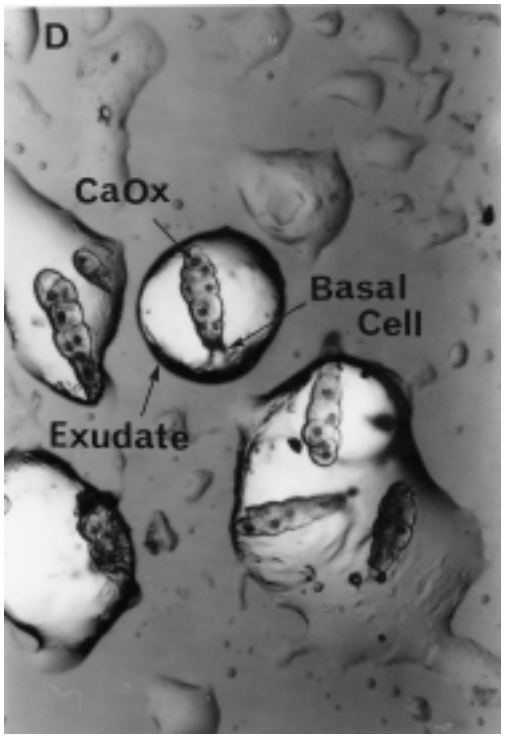


Background:

Exudating plant trichome glands are specialized tissues that extend from plant aerial surfaces. About 30% of higher plants have exudating glandular trichomes. Trichome exudate can consist of up to 30% of leaf dry weight, making glands of certain plants potential “factories” for molecular farming.

Depending on the plant species, trichome glands produce and exudate various biochemicals (often lipophylic, often terpenoids) to the leaf surface where they serve various roles for the plant (pest and pathogen resistance, temperature control, etc.).





The above photo shows an aphid walking on a leaf stained to reveal exudate. Note the accumulation of sticky exudate on its legs, etc. At the left is a light micrograph showing detached glands and exudate.

In the system we are studying (the experimental tobacco, T.I. 1068) glands produce up to 17% of leaf dry weight as exudate. The main components are diterpenes (about 70%) and sugar esters (about 25%).

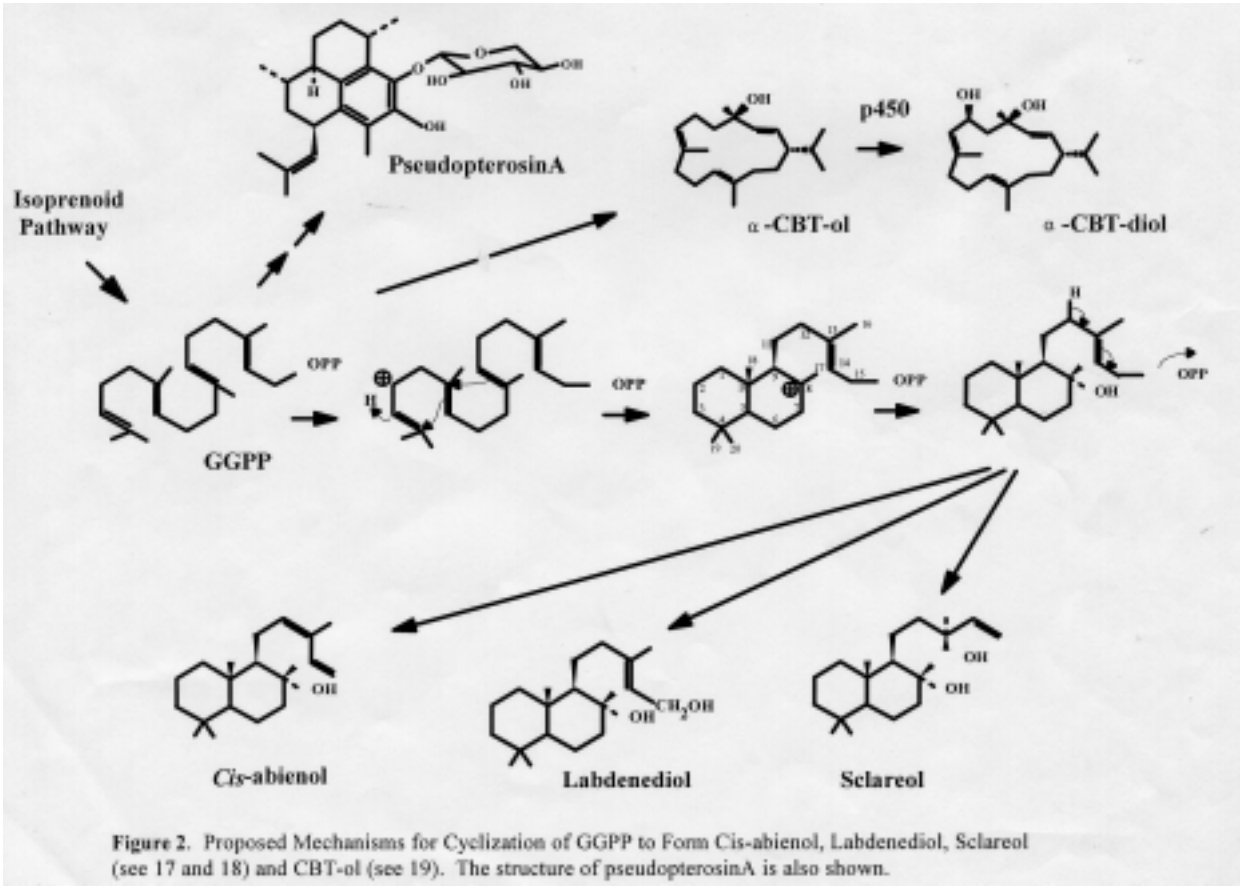
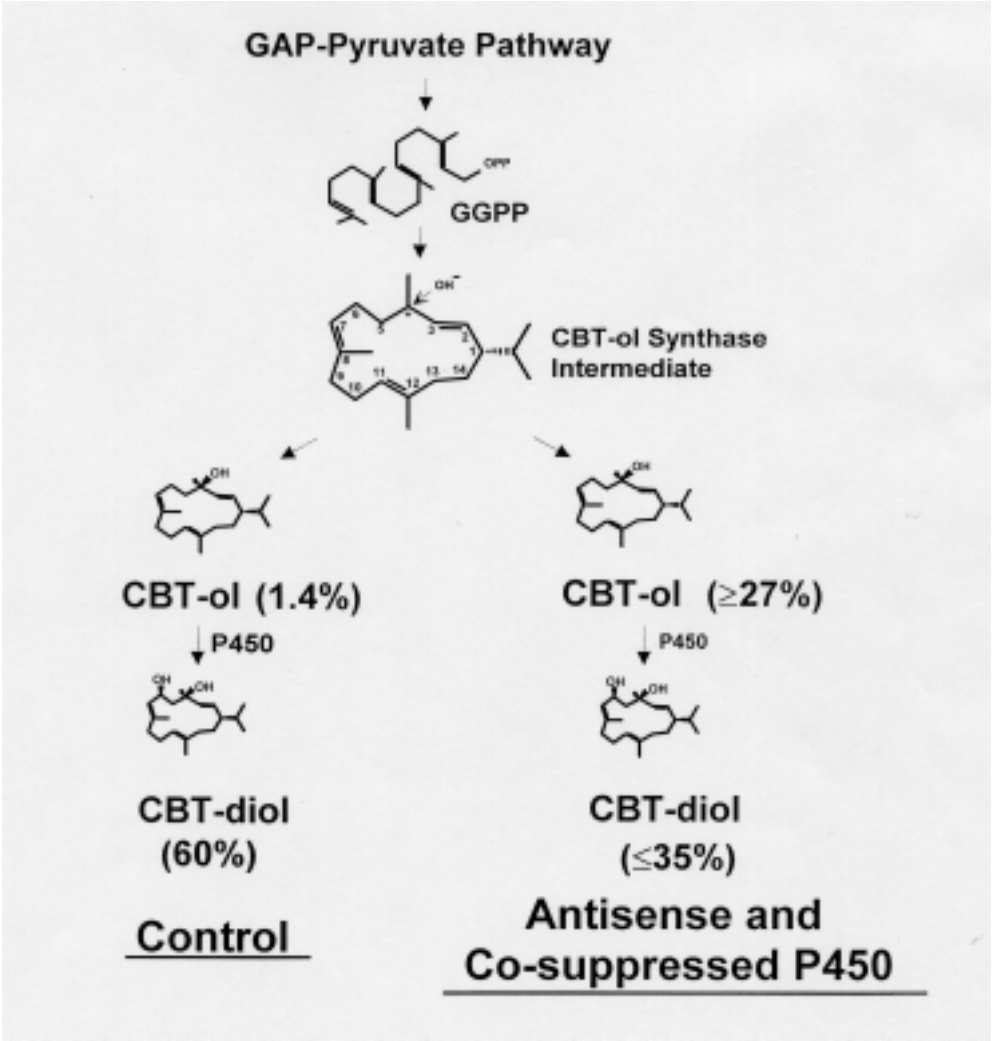


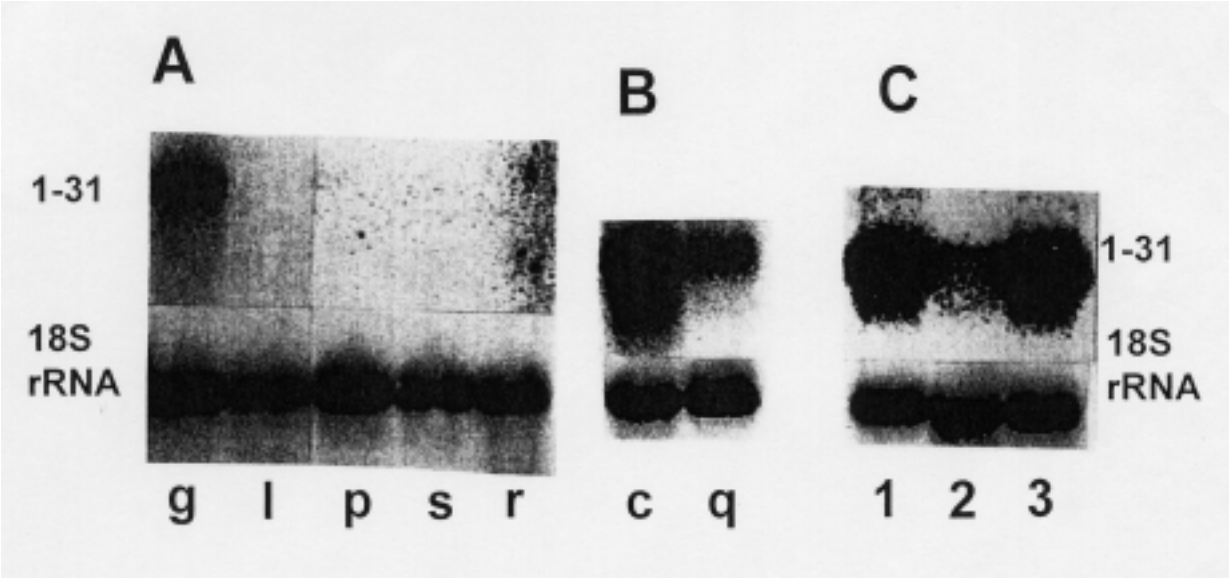
Figure 2. Proposed Mechanisms for Cyclization of GGPP to Form Cis-abienol, Labdenediol, Sclareol (see 17 and 18) and CBT-ol (see 19). The structure of pseudopterosinA is also shown.

Planta 197: 627-632 (1995), Plant Sci. 110: 1-10, 1995

We are studying carbon flow in diterpene biosynthesis to determine the plasticity and regulation of this pathway. Thus far, a “knockdown” approach (using antisense and co-suppression) has been very useful for assessing the function of one gland-specific gene, and for determining the ability of the system to accommodate an alteration in a terminal enzyme of the diterpene biosynthetic pathway (Nature Biotechnology 19: 371-374, 2001. This approach is currently being used (also now RNAi) to assess the functions of, and to manipulate two additional gkand-specificgenes.



The P450 gene is only expressed in trichome glands.



Northern analysis - 10 micrograms total RNA in each lane.

A: glands-g; l-leaf-minus-glands; p,s,r-petals, stem-minus-glands, roots, respectively. B: glands from a wild type plant-c; glands from a antisense plant. C: glands from a wild type plant-1; glands from a co-suppressed plant-2, glands from a non-suppressed, co-suppression plant-3.

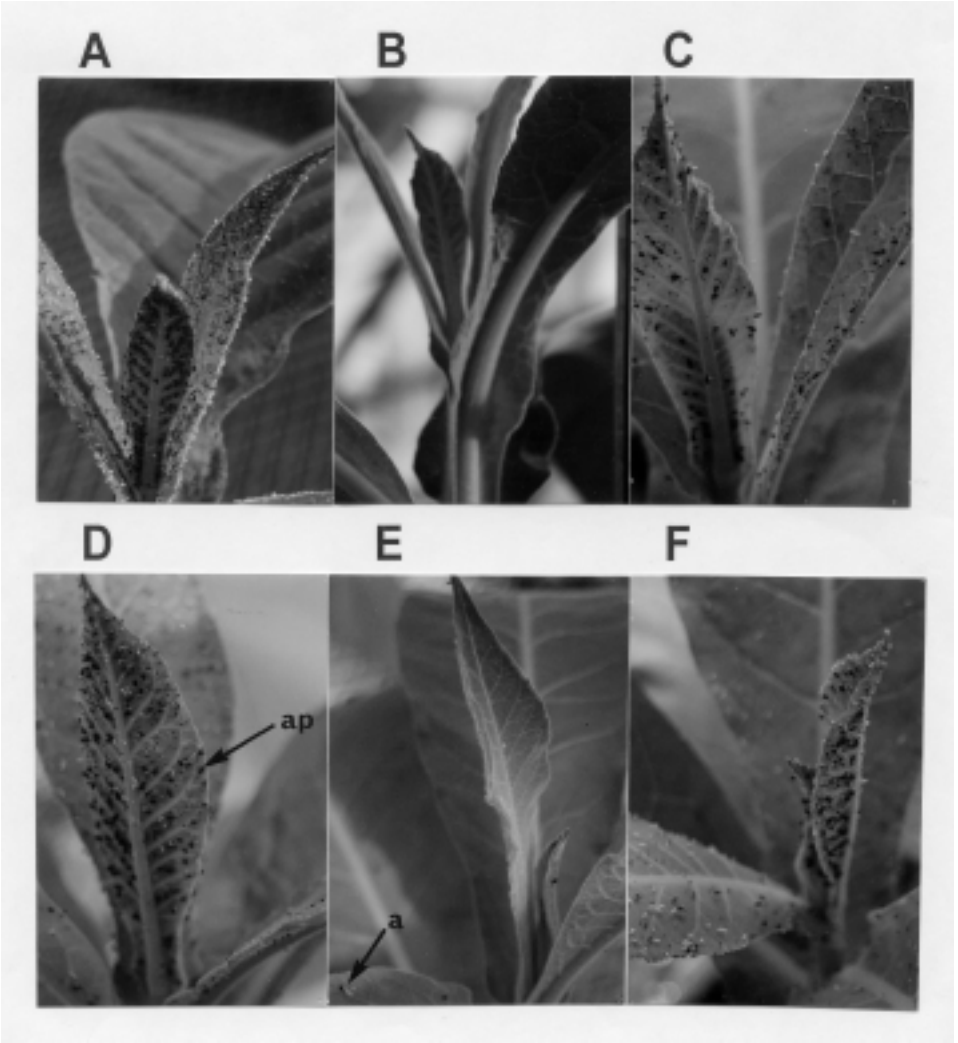
Using the diterpene P450 gene we have identified, we are comparing antisense, co-suppression, and RNAi to assess the most efficient “knockdown” method for determining the functions of other gland-specific cDNAs and genes we have isolated. We are also expressing one full-length, putative terpene cyclase gene in *E. coli* to determine its function.

P450	#PLANTS	#Q-like	%Q-like	ol/diol
anti-partial (1-31-2)	21	1	3.33	0.753
sense-partial (1-31-1)	29	0		
anti-full (FC)	29	5	17.2	2.13 ± 1.16
sense-full (FA)	28	5	17.9	3.09 ± 1.86
antiTSP-full (FB)	4	0	0	
senseTSP-full (FD)	23	0	0	
RNAi-partial				

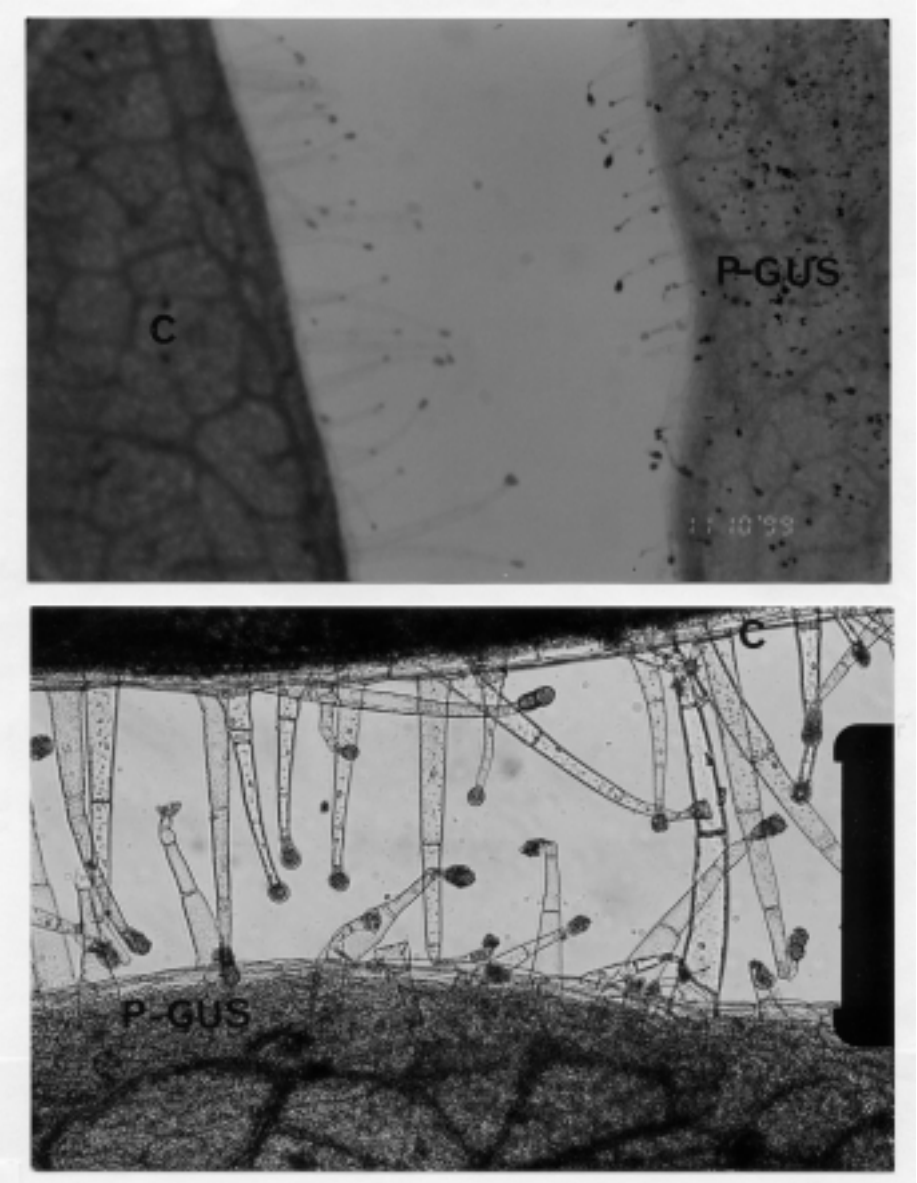
Wild type ol/diol is 0.02 - 0.03.

Aphid colonization of P450 suppressed plants is substantially reduced relative to controls. Consistent with this observation is the finding that exudates of P450 suppressed plants have higher toxicity to aphids than exudate from control plants (data not shown).

A- wild type plant; B- antisense plant; C- un-suppressed antisense plant; D- wild type plant; E- co-suppressed plant, F- un-suppressed, co-suppression plant.



The genomic clone of the trichome-specific P450 gene was isolated and used to define its promoter. This promoter (patent pending) was used to drive the GUS reporter gene, showing its trichome-specific nature. This promoter should be useful for expressing heterologous genes in glands to study metabolic engineering in glands, and for molecular farming using the trichome system. Light micrographs: c-control, P-GUS- promoterGUS expressing.



We are now:

- Investigating the functions of two putative terpene cyclase genes (one cloned).

- Preparing various tobacco systems for use as hosts to introduce genes that may modify exudate terpene composition.