Results Summary and Discussion

Anaerobic

- On the whole, plants treated to anaerobic treatment were similar to untreated controls. Anaerobic stress marginally increased shoot FW at fullflower stage and reduced root FW at goose-neck stage. All other measures of plant growth and morphology were not significantly different from controls.
- Galanthamine content was significantly increased in shoots at gooseneck stage and total shoot yields were increased both at gooseneck and fullflower. Bulb galanthamine content decreased significantly at full-flower stage. Overall total plant galanthamine yields were not shown to be significantly affected by the anaerobic treatment.
- These data indicate that exposure to low-oxygen conditions in the root environment, as could be caused by soil compaction, flooding or waterlogging, may cause enhanced galanthamine accumulation in aboveground organs at a detriment to accumulation in bulbs. However, anaerobic stress is unlikely to significantly impact on total galanthamine yields. These findings should be further validated in pot/field trials given that an artificial system was used in this investigation

Chitosan

- There was little evidence to suggest that foliar application of chitosan at concentrations up to 100mg/L stimulated growth, although marginal increases in sampling FW and shoot FW were observed at goose-neck stage.
- Chitosan significantly reduced galanthamine content and yield of bulbs 50mg/L treatment (gooseneck) and decreased shoot galanthamine content at 100mg/L dose. However, overall there was no significant effect on total galanthamine.
- These findings suggest that foliar application of chitosan is unlikely to be an effective mechanism to induce growth or alkaloid accumulation. The plant defence response pathways activated by biologically active chitosan polymers are most likely distinct from those which contribute to galanthamine accumulation.

Cold

- Exposure to intermittent and repeated chilling had a significant impact on growth and morphology of daffodil plants. Specifically, cold stress to roots, and especially shoots and roots combined, stunted FW growth, reduced shoot and root FW and plant/leaf height. Galanthamine content and yield was not significantly affected by cold stress treatments compared to untreated controls.
- These data demonstrate that cold stress is detrimental to crop yields although is insufficient evidence to demonstrate that API yields would be significantly altered as a result. Further studies should determine the outcomes of prolonged exposure to chilling or freezing conditions.

Drought

- Drought stress, induced by withholding of watering, had a dramatic effect on growth and morphology of daffodil plants in this study. Both drought stress regimes severely stunted growth as observed by all measures of plant growth and morphology,
- Drought dramatically reduced bulb, shoot and total galanthamine yields. Shoot galanthamine content was increased by drought regime 2. However, total shoot yields were reduced as a consequence of growth inhibition.
- It is shown that severe water deficit reduces daffodil growth and content of the API. Cultivation of daffodils on sites prone to drought should be avoided and soil management practices should aim to enhance soil water retention and availability. If possible, effective irrigation should be put into place in cases of drought sensitivity.

Harvest Regime

- Total plant FW, shoot FW and FW growth peaked at the fullflower stage. Bulb FW increased only during leaf senescence. Bulb Galanthamine content increased during flowering, shoot galanthamine decreased significantly following flowering and during leaf senescence. Bulb, shoot and total galanthamine yields fluctuated marginally during the growth period but were significantly higher than at planting stage.

- These findings confirm that galanthamine content and yield is highly dependent on developmental stage of the plants, and thus careful consideration should be taken in the selection of optimal harvesting times and post-harvest storage processes in order to maximise total extractable API.

Herbicides

- Treatment with sub-lethal doses of herbicides glyphosate and rimsulfuron severely reduced plant growth and morphology by fullflower stage and the effect was dosage dependant, as would be expected, although treatment with 2 µM glyphosate (gooseneck stage) marginally increased shoot growth.
- At fullflower stage, 50 µM glyphosate treatment increased shoot galanthamine content at, whilst 2 µM rimsulfuron reduced bulb content at fullflower. Overall, bulb, shoot and total galanthamine yields were significantly reduced as a consequence of growth inhibition.
- The results reveal that herbicide stress and injury, caused by treatment with broad spectrum systemic herbicides, does not to enhance overall galanthamine yield even when applied at sub-lethal doses.

Jasmonates

- Treatment with jasmonates severely reduced growth of plants in a dose dependant manner. In particular, plants were sensitive to JA and meJ treatment which caused stunting of plant growth and morphology.
- Treatment with meJ significantly increased shoot alkaloid content at 5mM. However, overall, treatment with JA or meJ significantly reduced shoot galanthamine yields as a

consequence of shoot growth inhibition. Total galanthamine levels were not significantly different across the treatments tested.

- The results demonstrate that daffodil plants are sensitive to jasmonates, known plant defence inducing hormones, and that galanthamine content can be increased by exogenous application of these chemicals, at least in shoot tissues. This is consistent with the hypothesis that galanthamine and other daffodil alkaloids play a role in defence against predators. At the same time, however, jasmonates reduce allocation to vegetative growth and cause stunting of plants and thus there is no net increase in API. However, since timing of application may be an important factor, further trials should determine whether late-season treatment with jasmonates, at the time when plant biomass is already at its highest (i.e. full-flower), would also stimulate alkaloid accumulation.

Metals

- Plants grown in nutrient solutions containing high concentrations of soluble aluminium and manganese metals did not exhibit symptoms of metal toxicity or nutrient imbalance, nor was plant growth and size adversely affected on the whole. There was no convincing effect of either metal treatment on galanthamine content or yields.
- The data suggest that daffodils plants show tolerance to high levels of bioavailable trace elements present in the root environment, such as soluble manganese and aluminium which are known to accumulate in acidic soils. The experiment would need to be continued for several seasons to demonstrate this convincingly.

Nitrogen

- High nitrogen treatment (16mM total N) significantly reduced shoot FW, plant FW compared to controls. Additionally, plants grown in nitrogen deficient media showed a significant reduction in FW, FW growth and shoot/leaf FW, although treatment at 8mM N with an equivalent Cl concentration to the 0mM treatment (N Cl control), had a similar effect, confounding these findings. Galanthamine content of bulbs and shoots was unaffected, as was total galanthamine yield.

- There was insufficient evidence to support the hypothesis that nitrogen availability has a positive influence on alkaloid accumulation in daffodils, at least in the short term. However, given the bulb acts as a nutrient store/reserve, nitrogen deficiency may only be manifested after several growing seasons under low N conditions. Application of N fertilisers to increase N availability should be done with caution, as there appears to be no net benefit of increasing N supply, and in some cases may be deleterious to crop yields.

Nutrient

- Plants grown in nutrient deficient solution exhibited significantly reduced FW, FW growth, shoot/leaf FW compared to controls. Plants grown in nutrient solution containing double concentrated macro and microelements showed somewhat increased FW growth at fullflower stage, yet decreased FW growth at gooseneck stage, compared to controls, and such these data are inconclusive.
- Bulb galanthamine content and shoot galanthamine yields were significantly reduced in plants grown in nutrient deficient conditions. In addition, total galanthamine yield was found to be significantly decreased at gooseneck stage compared to controls. Galanthamine content was largely unaffected by growing plants in double concentrated nutrients.
- It is suggested that availability of total nutrients (macronutrients and trace elements) is an important factor in galanthamine accumulation in daffodil plants, whereby cultivation under conditions that may lead to nutrient depletion would negatively impact on content and yield of the API.

Ozone

- There were no significant effect of the ozone treatments (concentrations 19.0, 62 and 66.6 ppb) on plant growth and morphology, compared to controls grown at current background uplands levels (concentration 42.9 ppb).
- Bulb galanthamine and total galanthamine yield was found to be significantly increased at 62.0 ppb. However, there was no significant difference at 66.6 ppb nor at the lower dose of 19.0 ppb. There data are inconclusive with regard to understanding the relationship between atmospheric ozone and API content of daffodils.

pH

- There was no observed effect of pH on plant growth and morphology and similarly no significant differences in galanthamine content and yield across the treatment range (pH5-8). However, the experimental period may be too short for any effects of pH to have been manifested. The data suggest that management of soil pH is not an important consideration in terms of contribution to the API, at least in the short term.

Phosphorus

- Shoot growth was significantly reduced at full flower stage when daffodils were grown in P deficient nutrient solution (0mM phosphate), compared to controls (1mM phosphate).
- Shoot galanthamine yield was significantly reduced at goose-neck stage in P deficient treatment, compared to controls. In contrast, bulb galanthamine and total galanthamine were significantly reduced under high P treatment, at full-flower stage.
- There is unlikely to be a significant impact on P availability on galanthamine content of plants, unless plants are grown in very P deficient or P rich soils. Excessive use of P fertilisers should be avoided and soil management practices should avoid depletion of

available P as these would both negatively impact content and yield of the API; these effects would be exacerbated after repeated growing seasons.

Potassium

- On the whole, growth and morpholology of daffodil plants grown in potassium (K) deficient (0mM) or K supplemented (8mM) conditions was not significantly different to controls (4mM), although leaf FW was marginally reduced at fullflower stage.
- There was no response to K availability in terms of % DW content of galanthamine in bulbs and shoots, although high K treatment significantly reduced overall shoot yield at gooseneck stage.
- It is suggested that potassium availability is not an important factor affecting content and yield of the API, at least at the concentrations tested in this study. However given that the daffodil bulb acts as a nutrient store/reserve, K deficiency may only be manifested after several growing seasons in K depleted media, and thus this is not apparent from this study. It is suggested that excessive use of K fertilisers or cultivation in K rich soils should be avoided as this may reduce accumulation of the API.

Salicylates

- Galanthamine content and yield was shown not to be significantly affected by either SA and meS at the concentrations tested, although high concentrations of SA (5mM) reduced plant growth and morphology. The findings indicate that exogenous application of salicylates would be an ineffective strategy to enhance content and yield of the API. The accumulation of galanthamine appears to be stimulated by biological pathways distinct from those activated by SA, i.e. jasmonates.

Salinity

- Exposure to saline conditions (50 and 100mM NaCl) induced salinity stress in daffodil plants as observed by reduction in FW, FW growth, shoot/root FW and morphololgy and the response was dose dependent.
- Salinity stress dramatically reduced bulb galanthamine content yet increased shoot galanthamine, at the fullflower stage. Shoot galanthamine yield remained comparable to untreated controls, however bulb yield was significantly reduced. Overall, total yields of the API were significantly reduced by both salinity treatments.
- Daffodil plants appear to be very salt sensitive indicating low tolerance to osmotic and ionic stress. Cultivation on saline/salt-affected soils should be avoided and soil management practices should ensure salinization does not occur, as this would negatively impact on content and yield of the API.

UV

- Plant growth under regimes of elevated UV-B light exposure (0.3-1.0 Wm^{-2} absolute irradiance) was not significantly different compared to background controls (0.05-0.1 Wm^{-2}) in this investigation. Galanthamine content of bulb and shoot tissues was not significantly affected by UV-B treatment nor were total yields affected.
- Data from this study do not support the hypothesis that exposure to elevated UV-B radiation is a significant factor impacting on content and yield of the API. Furthermore, daffodil plants appeared relatively tolerant to high UV-B levels, at least at the conditions tested in the greenhouse, although this may not exactly represent spectral conditions in the field.

Wounding

- Wounding stress consistently reduced growth and morphology of daffodil plants across all treatments tested. Wounding both increased and decreased galanthamine content of bulb and leaf tissues, depending on the context of the wounding stimulus applied. However, all wounding treatments significantly reduced overall shoot galanthamine yields, but total yield were not significantly different.

- In particular, wounding induced by leaf crushing decreased bulb galanthamine content and yield yet increased shoot content; overall shoot yield was reduced due to inhibition of shoot growth. In contrast, leaf cropping reduced both shoot galanthamine and shoot alkaloid yield, possibly as a result of removal of available metabolite pools in the plant.
- The results indicate that exposure of daffodil crops to wounding stimuli i.e. damage by adverse weather, predators, machinery or livestock, has a deleterious effect on crop yields. Importantly, the data suggest that the practice of leaf 'cropping' as a harvest strategy would be unsuitable for repeated harvests within a growing season, as diminishing returns would be a factor.