Figure 4 Symptom-bearing and symptomless *Nicotiana benthamiana* plants inoculated with Can-S.

10-35% of the number of local lesions present on comparable control plants but ELISA values were 65-94% of controls (Table 1). This suggests that these transformants may have some resistance to inoculation with RBDV but little or no resistance to its multiplication once plants are infected.

This work has demonstrated the effectiveness of transformation with the RBDV-CP gene or -pol gene sequences to induce resistance to RBDV infection in *Nicotiana* species. Furthermore, it demonstrates that this resistance can be conferred whether the CP gene is in a translatable or non-translatable form, and whether it occurs in the sense or anti-sense orientations. These results are encouraging and, if equally successful when applied to *Rubus* plants, offer the prospect of providing possibly the best means of protecting future *Rubus* crops against this difficult-to-control pathogen.

The increasing importance and control of wingless weevils as pests in temperate World horticulture

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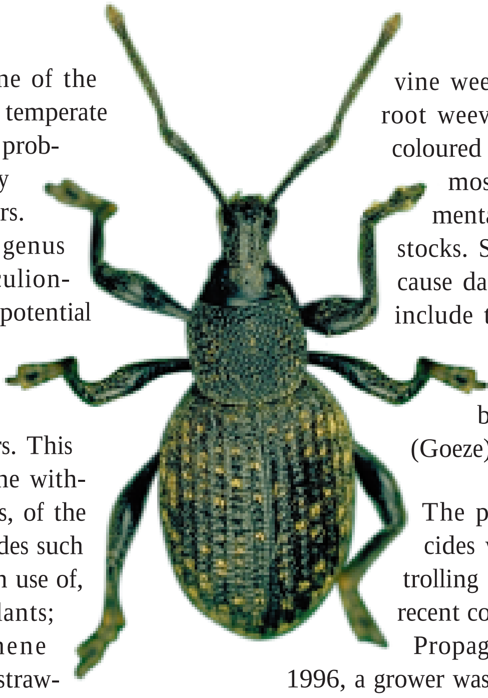

Wingless weevils are now one of the most troublesome pests of temperate horticulture, causing considerable problems to fruit, ornamental nursery and forest nursery stock producers. Wingless weevils belong to the genus *Otiorhynchus* (Coleoptera: Curculionidae) and have been recognised as potential pests of a wide range of crops for many years, but they have become particularly troublesome in horticulture in the last 15-20 years. This follows, amongst other factors, the withdrawal, on environmental grounds, of the persistent organochlorine insecticides such as aldrin and DDT; the increase in use of, and trade in container grown plants; and the reliance on polythene mulches, especially in soft fruit (strawberry and blackcurrant) production. Three species, the vine weevil (*O. sulcatus* (F.)), strawberry root weevil (*O. ovatus* (L.)) and the clay-coloured weevil (*O. singularis* (L.)), are the most damaging to fruit, hardy ornamentals and in commercial tree nursery stocks. Several other *Otiorhynchus* species cause damage to soft fruit crops and they include the red-legged weevil (*O. clavipes* (Bonsdorff)), and *O. rugifrons* (Gyllenhal)) and the rough strawberry weevil (*O. rugosostriatus* (Goeze)).

The persistent organochlorine insecticides were particularly effective in controlling this group of insects. Indeed, at a recent conference of the International Plant Propagators Society in Cork in August 1996, a grower was quoted as saying 'Life after aldrin is difficult and expensive....Control of vine weevil is

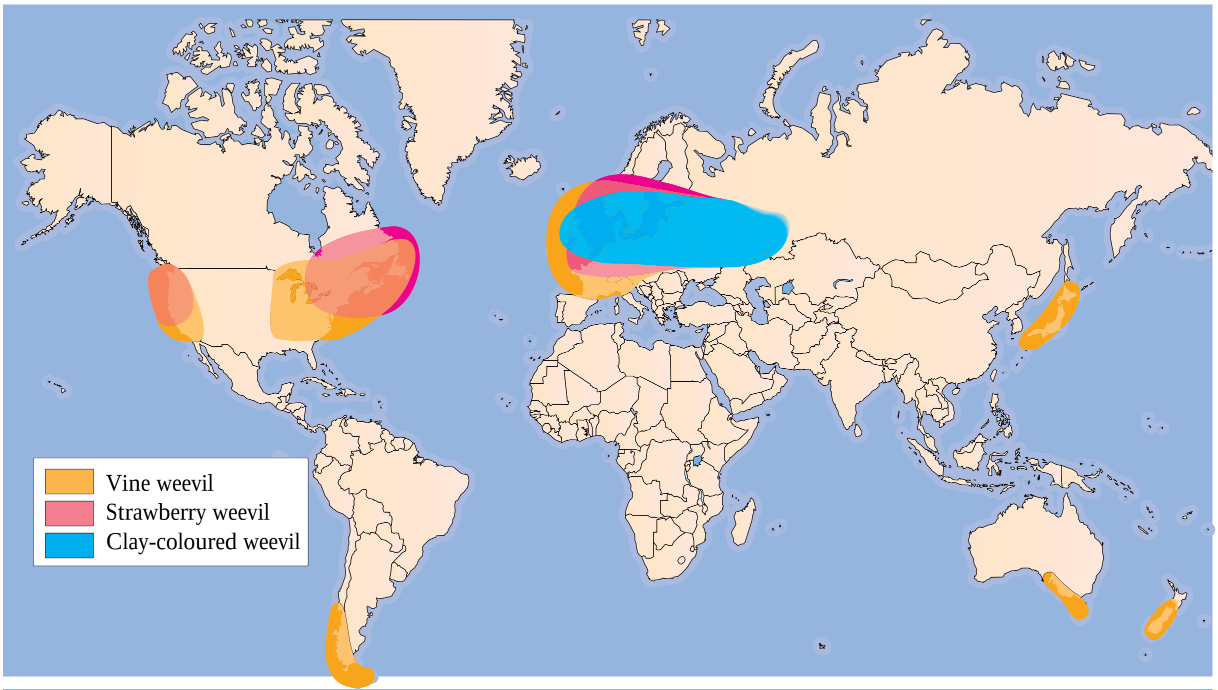

Figure 1 The approximate geographic distribution of the main temperate Otiorhynchid weevil species (after Moorhouse *et al*, (1992). *Annals of Applied Biology* **121,** 431-454)

very, very difficult....It takes number one priority on our nursery.'[1]

The genus *Otiorhynchus* is thought to be of European origin but *O. sulcatus*, in particular, is now widespread in most temperate regions of the World (Fig. 1). *O. singularis* is largely confined to north western Europe, whereas *O. ovatus* is widespread in both Europe and North America.

Biology of wingless weevils As their name implies, wingless weevils cannot fly because their elytra (wing cases) are fused but they can disperse easily by walking, as contaminants sheltering in pots or crates used to transport plants or produce, and as eggs or larvae in the compost of containerised plants. The most numerous wingless weevil, *O. sulcatus*, has one generation per year, but there may be some overlap between the generations. Adult weevils are all parthenogenic females (Fig. 2a), no males being found, and some species are relatively large (7-11 mm). Adult weevils feed at night soon after emergence, hiding in the soil or within dense foliage during daylight hours. After emergence from the pupal stage, all weevils need a period of intensive feeding before they start to lay eggs and foliar feeding becomes most noticeable at this time. The number of eggs laid varies considerably but on average, each female can lay several hundred. Although only a small proportion of the eggs may survive to complete their development, this is sufficient to allow for a rapid increase in the population. The larvae (Fig. 2b) of all the species feed on the roots of their host plants, but the response to feeding varies

a)

b)

Figure 2 a) Adult vine weevil b) Clay-coloured weevil larva.

Figure 3 Clay-coloured weevil feeding damage to raspberry plants.

considerably. Single larvae can kill very susceptible plants, whereas large populations may be tolerated on others e.g. clay-coloured weevil larvae appear to cause little root damage to raspberry. The different species may have different larval feeding preferences, e.g. vine weevil larvae tend to feed on roots close to the surface, whilst clay-coloured weevils have been observed feeding on raspberry roots at depths below those reached by conventional pesticide application techniques.

Laboratory observations indicated that adult clay-coloured weevils were able to survive for up to 3 years. *O. singularis* adults emerge from the soil in May and June and feed after dark on bursting raspberry buds and developing fruiting laterals (Fig. 3), but rarely feed on fully developed raspberry foliage. Laboratory studies indicate that they avoid feeding on damaged raspberry foliage, probably because of the release, or induction, of anti-feeding compounds[2].

Host ranges of Otiorhynchid weevils The vine weevil is extremely polyphagous, with larvae and adults found feeding on many perennial and semi-perennial plant species grown in both glasshouses and in the open. The hosts range from maidenhair ferns, through perennial fruit crops (e.g. strawberry, grape vine and blackcurrant), to perennial ornamentals such as *Taxus* and *Rhododendron*. The host ranges of the other Otiorhyncids are thought to be narrower, e.g. clay-coloured weevils cause extensive feeding damage to raspberry and girdle stems of newly planted trees or feed on buds and grafts of apple and pear.

Control strategies present and future *Present control* The current range of commercially available insecticides is not particularly effective against adult weevils and few are effective against larvae. Some success in controlling larvae in nursery stock has been achieved by using a slow release granular formulation of chlorpyrifos and a microencapsulated formulation of fonofos. However, to be fully effective, all the compost in a pot needs to be treated with a precise dose of insecticide. When properly incorporated into compost, these products have been shown to be effective for up to two years. These products are unlikely to be used to control field infestations of vine or other weevil because of difficulties of incorporation into the soil around the growing plants and the relatively high cost involved.

In recent years, much effort has been given to develop a biological control strategy for wingless weevils. Some success has been achieved using the entomopathogenic nematodes (*Steinernema* and *Heterorhabditis* spp.) against larvae. These nematodes work well against vine weevil larvae in protected cultivation, but have proved to be less reliable under field conditions, especially when soil temperatures are low. Several centres are currently striving to isolate and develop strains of nematodes which will be effective at temperatures below 10^{o} C. Similarly, the insect parasitic fungi, *Metarhizium* sp. and *Beauveria* sp. have been investigated and shown to be effective under some conditions.

Future control Control of wingless weevils will remain problematic in the future. It is unlikely that any one control strategy will prove to be effective. Populations of weevils will have to be managed in such a way as to reduce the risk of spread from infected areas into ‘clean zones’. The fact that wingless weevils do not fly could be exploited by the use of physical barriers, either on their own, but more likely in combination with adhesive strips. Where possible, clean land should be selected for growing susceptible crops or a lengthy period of crop rotation practised.

Suitable monitoring systems for the presence of adult weevils need to be developed to warn the grower of the problem at an early stage of infestation. The use of appropriate control measures at an early stage of infestation will be more effective than trying to manage large damaging populations. Monitoring of the insect, use of appropriate insecticides, and selection of growing sites all form part of an Integrated Pest Management (IPM) strategy for weevils. In addition, recent research at SCRI has show that the insertion of the cowpea trypsin inhibitor (CpTi) gene from the tropical legume (*Vigna unguiculata*) into strawberry, may protect plants from vine weevil attack in glasshouse trials. Field trials are currently underway to establish the effectiveness of genetically modified plants when challenged by weevil attack under field conditions[3]. If these prove successful, there is now the opportunity to modify other high value fruit crops to protect them from vine and other wingless weevil damage.

Natural plant resistance to wingless weevils Much effort has gone into finding sources of natural plant resistance to vine weevils. Resistance has been identified in the beach strawberry (*Fragaria chiloensis*) from the Pacific Northwest of America and in some *Rhododendron* stocks, particularly the lepidote type. Observations on the feeding behaviour of clay-coloured weevil on raspberry have shown that this insect will avoid mature or damaged raspberry leaves and will preferentially feed on stems, bud or developing canes. It is thought that they have developed this feeding strategy to avoid potential anti-feeding compounds that may be present in raspberry leaves. In a feeding study where weevils were exposed to healthy and wounded raspberry leaves in complete darkness (video images obtained using an infra-red video technique), adult weevils rapidly moved away from the wounded leaves to shelter under the undamaged healthy leaves[2].

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