CLASSICAL AND MOLECULAR APPROACHES IN BREEDING FOR RESISTANCE TO INSECTS IN ORNAMENTALS.

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INTRODUCTION

The extent to which insecticides are needed is influenced by the level of resistance in the host plant. Plant breeders control this level through the choice of parents and selection procedures. If plants are exposed to insects during selection, resistance can be recognised and selected for. When, on the other hand, plants are fully protected from insects, e.g. through application of insecticides, the resistant phenotype goes unnoticed. In such a setting, genes for resistance have no selective advantage and may be lost.

CLASSICAL APPROACH

The practice in breeding ornamentals has been to protect plants from insect attack during selection. Still, cultivars developed under such regimes show large differences in herbivory by insects. We exposed carnations, unselected for resistance to *Frankliniella occidentalis*, the western flower thrips, and observed large differences in damage imposed (Fig 1). Similarly chrysanthemums, unexposed to *Liriomyza trifolii* in the selection process, show large differences in herbivory by the leafmining larvae (Fig. 2).
Both examples show that genetic variation for resistance is still available in modern cultivars, ready to be utilised before reverting to wide crosses or gene transfer technology. Screening methods, to detect insect resistance, are described in Smith et al. 1994

Fig. 1 Eight single flower plants from each cultivar of carnation were exposed to western flower thrips in a choice setting. Thrips fed and multiplied on the flowers which subsequently collapsed. The number of days (5 to 21) to destruction of the flowers is presented for each cultivar.

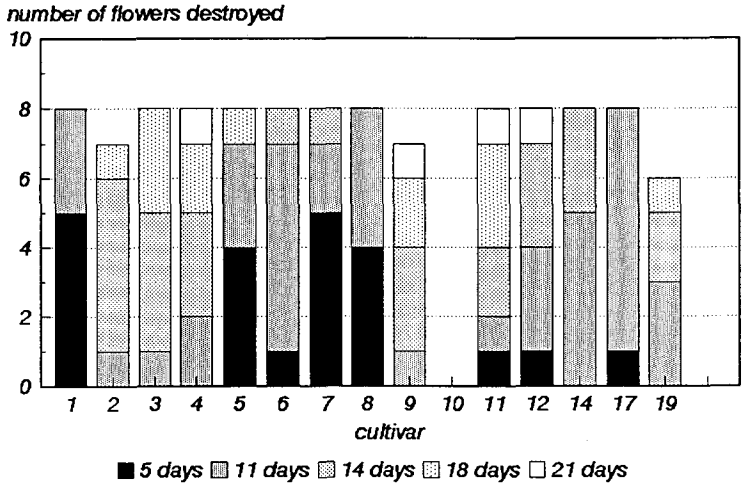
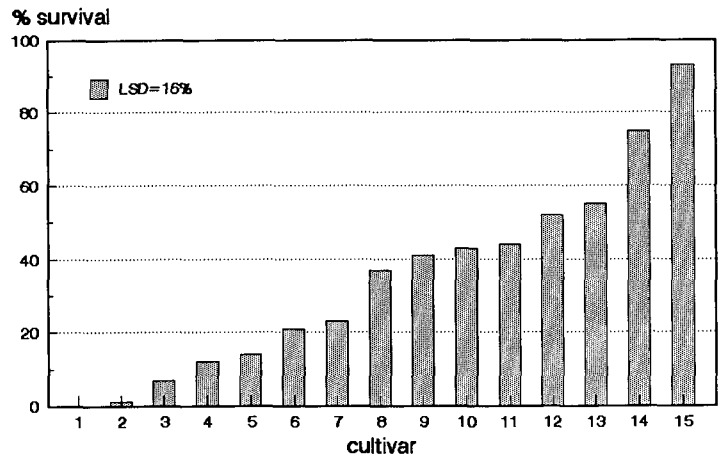


Fig. 2 Adult flies of *Liriomyza trifolii* laid eggs on *Chrysanthemum morifolium* in a no-choice situation. The eggs hatched and the survival of the larvae feeding inside the leaves of 15 cultivars was determined (De Jong & Van de Vrie, 1987)

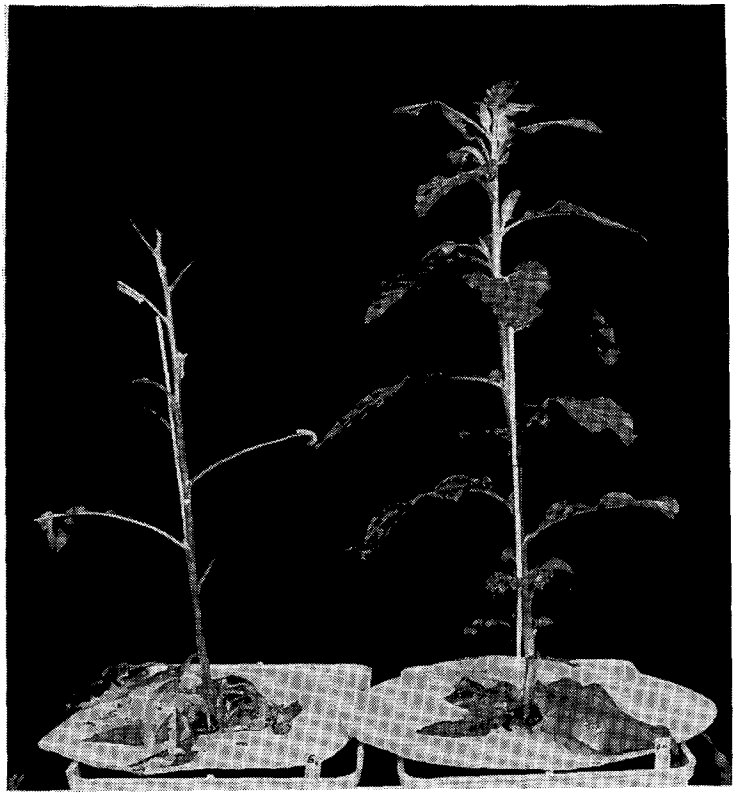


MOLECULAR APPROACH

Where resistance in the hostplant falls short of requirements, the gene pool of other species may be employed. We are presently introducing two classes of genes that interact with insect development: Crystal protein genes (*cry* genes) from *Bacillus thuringiensis* and Proteinase Inhibitors (PI genes) from potato.

- Crystal protein genes. A large number of crystal protein genes is available that are very specific for larvae of target insects. The *cry* genes, being of bacterial origin, are difficult to express in plants, even when provided with an efficient plant promotor. Expression in plants is raised by changing wild type codons to plant-like codons without altering the encoded amino acid sequence. Insect-resistant plants have been obtained through the introduction of *cry* genes. Figure 3 shows the effect of the codon-modified *cryIC* in tobacco on herbivory by *Manduca sexta*. This gene has now been introduced into chrysanthemum (De Jong et al. 1994) to obtain resistance to *Spodoptera exigua*.
Crystal proteins eaten by an insect are processed in the midgut. The toxic part attaches to receptors on the epithelial cells, thereby desintegrating the membrane, leading to death. Insects may acquire resistance by preventing the protein to attach to the membrane through mutation of the receptor site. A possible strategy to prevent development of resistance would be to simultaneously employ different gene classes in resistance management strategies. (Van der Salm et al. 1994; Bosch et al. 1994). A series of simultaneously occurring mutations in the insect is than required to obtain resistance.

Fig. 3 Plants of 'Samsun' tobacco five days after inoculation with 10 larvae of *Manduca sexta*. The control plant is reduced to stalk. The larvae on the *cryIC* producing plant began to feed and died.



- Proteinase Inhibitors. Inhibitors of insect gut proteinases are a second class of anti-insect genes. PI's are proteins produced by plants which bind to proteinases of insects to form an inactive complex. Such complexing inhibits digestion of food and results in reduced growth of the insect. The effect of Proteinase Inhibitors is less dramatic than that of the toxic crystal protein genes but the spectrum is thought to be broader.
Each insect employs specific proteinases for digestion and the approach that we have chosen is to identify the proteinases of our target insects (*Spodoptera*, leafminer and thrips). The pH of the digestive system is also determined as the association of the inhibitors with the respective proteinases is pH dependent. After identification a matching inhibitor protein is selected and tested in vitro by exposing the midgut contents to a range of inhibitors to measure the inhibitory activity. Feeding trials, in which an inhibitor is added to the diet are also employed to study the effect on growth or reproduction of the insects. Finally the corresponding gene is inserted in the plant. No problems are expected with transcription and translation since the genes are of plant origin. *Spodoptera* was shown to employ serine proteinases and leafminers and thrips mainly aspartate and cysteine proteinases. PI-1 (serine), PI-2 (serine) and PI-4 (cysteine) have now been introduced into chrysanthemum.

The first successful use of proteinase inhibitors was reported by Hilder et al. 1987. They introduced cpTI in tobacco and obtained resistance against lepidopteran insects. Potato proteinase inhibitor II gave resistance to *Manduca sexta* (Johnson et al. 1990) and *Chrysodeixis eriosoma* in tobacco but not to closely related *Spodoptera litura* (McManus et al. 1994) illustrating that effect of PI-II depends on the insect that is challenged. Next to sensitivity of the insect, the endogenous production of inhibitors by the plant also plays a role. Wounding of plants induces the synthesis of proteinase inhibitors (Jongsma et al. 1994). Upon wounding, tobacco plants express a level of PI that, within a day, exceeds the level produced by the transgene. It seems therefore especially attractive to introduce heterologous genes for PI's that the host plant cannot induce and that extends the range of inhibitors available to the host.

REFERENCES

Bosch D, Schipper B, Van der Kley H, De Maagd RA. 1994, Recombinant *Bacillus thuringiensis* crystal proteins with new properties: Possibilities for resistance management. Biotechnology 12:915-918.

De Jong J, Mertens MMJ, Rademaker W. 1994, Stable expression of the GUS reporter gene in chrysanthemum depends on binary plasmid T-DNA. Plant Cel Reports 14:59-64, 1994

De Jong J, Van de Vrie M. 1987, Components of resistance to *Liriomyza trifolii* in Chrysanthemum morifolium and Chrysanthemum pacificum. Euphytica 36:719-724

Hilder VA, Gatehouse AMR, Sheerman SE, Barker F, Boulter D. 1987, A novel mechanism of insect resistance engineered into tobacco. Nature 330:160-163

Johnson R, Narvaez J, An G, Ryan CA. 1990, Expression of proteinase inhibitors I and II in transgenic tobacco plants: Effects on natural defense against *Manduca sexta* larva. Proc. Natl Acad Sci USA 86:9871-9875

Jongsma MA, Bakker PL, Visser B, Stiekema WJ. 1994, Trypsin inhibitor activity in mature tobacco and tomato plants is mainly induced locally in response to insect attack, wounding and virus infection. Planta 195:29-35

McManus MT, White DWR, McGregor PG. 1994, Accumulation of a chymotrypsin inhibitor in transgenic tobacco can affect the growth of insect pests. Transgenic Research 3:50-58

Smith CM, Khan ZR, Pathak MD. 1994, Techniques for evaluating insect resistance in crop plants. ISBN 0-87371-856-9, CRC Press, pp320

Van der Salm T, Bosch D, Honée G, Feng L, Munsterman E, Bakker P. Stiekema WJ, Visser B. 1994, Insect resistance of transgenic plants that express modified *Bacillus thuringiensis cryIA(b)* and *cryIC* genes: a resistance management strategy. Plant Molecular Biology 26:51-59.