ENDOPHYTES OF APPLE AND KIWIFRUIT

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Endophytic fungi cause apparently asymptomatic infections entirely within the tissue of their plant hosts. Some of these seemingly benign fungi are mutualistic symbionts within the host, conferring resistance against insect pests, eg. *Acremonium* endophytes of grasses (Clay 1988), *Rhabdocline parkeri* protecting douglas fir against attack by *Contarinia* larvae (Sherwood-Pike *et al.* 1986), and *Gloeosporium quercinum* and *Kabatiella apocrypta* attacking gall-forming insects on oak leaves (Butin 1992). Although endophytes have been found to be widespread in many plants, most investigations into their occurrence have involved trees and shrubs of north-temperate regions (eg. Boddy and Griffith 1989). There have been no reports of work on endophytes within woody plants of horticultural importance.

The possibility of utilising naturally occurring fungal endophytes as insect feeding deterrents in apple and kiwifruit was explored. Such an approach could lead to a reduction in chemical residues through the use of fewer pesticides in these crops. The survey was intended to identify natural endophytes which could potentially be genetically manipulated to introduce, and maintain within the plants, genes for insect feeding deterrents.

Apple (Braeburn) was sampled from three HortResearch orchards in Auckland and Hawkes Bay, and kiwifruit (Hayward) from a HortResearch orchard in Auckland and two commercial orchards in the Bay of Plenty. From each orchard samples were taken from five randomly chosen plants in a single block. The blocks were sampled three times for tissue of different ages: 12- and 24-month-old xylem and bark tissue was sampled in July 1992; 4-month-old xylem, bark and leaf (petiole, midrib and lamina) tissue was sampled in December 1992; 8-month-old xylem, bark and leaf tissue was sampled in March 1993. Five different plants were chosen in each sampling. For each sample two branches were removed from each of the five plants and from each branch 10 approximately 10 mm square pieces of each tissue type were excised 24-48 h later. The symptomless tissue pieces were surface sterilised (95% ethanol 1 min, sodium hypo-chlorite solution containing 4-5% available chlorine 3 min, 95% ethanol 30 s), washed in sterile water, plated onto Difco malt extract agar in petri dishes, and incubated at 18-20°C under near UV lights with a 12 h photoperiod. These were examined at intervals up to 6 weeks. Any fungi which could not be identified directly from the original isolations were transferred to Difco potato-dextrose agar. Isolations were recorded as negative if no fungus had grown from the tissue pieces within 6 weeks. A generalised linear model with logit link and binomial errors was fitted to the percentage of tissue pieces infected in each group of 10 pieces taken from each branch sampled:

$\text{logit}(p) = \text{const.} + \text{tissue type} + \text{host} + \text{tissue age} + \text{tissue age}^2 + \text{orchard} + 2\text{nd order interactions}$, where p is the proportion infected.

An estimate of error using the orchards as replicates was calculated.

In comparison with other studies on fungal endophytes within woody plant tissue, a low number of xylem tissue pieces from the apple and kiwifruit plants surveyed were inhabited with fungi (Table 1). A species of *Leptodothiorella* was isolated from the xylem of several apple trees at orchards in Kumeu and Havelock North. A similar fungus sometimes fruits on the cut stems of apple trees (A. Spiers, HortResearch, pers.

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comm.). B.C. Sutton (CAB International) confirmed the identity of these isolates, and in his examination noted the occasional occurrence of *Phyllosticta*-like conidia. Several *Phyllosticta* species are known to have *Leptodothiorella* spermatial states, and species of this genus have often been reported as endophytes in other studies (Boddy and Griffith 1989), although more commonly from leaves than from woody tissue. From kiwifruit isolations *Microdochium* sp., *Phialophora* sp., *Phoma*-like spp., and *Septoria* sp. may be endophytic in wood. Although none of these species were isolated consistently, all were found only in wood, reducing the likelihood of their being chance contaminants from the bark surface.

The biology of these apparently endophytic isolates deserves to be explored. Any that can be inoculated into living plants to form long-term, asymptomatic, stable populations within the wood may have potential for development as biocontrol agents, or as vectors of biological control agents.

In the leaves and bark of both plant species infection levels in all tissue types increased with tissue age. Within leaves, petioles were most highly infected, followed by the midribs and the leaf lamina (Table 1).

TABLE 1: Infection levels in symptomless apple and kiwifruit tissue (percentage of sampled tissue pieces infected, average from the three orchards sampled for each host).

Host	Tissue type	Tissue age				LSD ^{ad}
		4 month	8 month	12 month	24 month	
Kiwifruit	Xylem	6	9	7	18	29
	Bark	35	69	92	98	29
	Leaf petiole	79	93	-	-	17
	Leaf midrib	58	78	-	-	30
	Leaf lamina	21	52	-	-	30
	LSD ^{ab}	32	30	15	28	
Apple	Xylem	1	2	12	10	15
	Bark	4	56	83	69	34
	Leaf petiole	31	78	-	-	37
	Leaf midrib	21	46	-	-	37
	Leaf lamina	3	12	-	-	22
	LSD ^{ac}	33	43	21	30	

^a Approximate LSD for $P < 0.05$ (LSD for $P < 0.05$ on the logit scale was 2.0 for kiwifruit and 2.5 for apple).

^b LSD refers to columns of kiwifruit data for each tissue age.

^c LSD refers to columns of apple data for each tissue age.

^d LSD refers to rows of data for each tissue type.

Most of the fungal species found were present in both plant species, but their relative frequencies differed. *Alternaria alternata*, the most common species in apple tissue (62% of petiole tissue pieces infected), was present at only low levels in kiwifruit (5%). *Phomopsis* spp. (80% petiole tissue pieces infected) and *Cladosporium cladosporioides* (29%), the two most common species in kiwifruit, were present at only low levels in apple (both species isolated from 1% of petiole tissue pieces sampled).

Full sets of results listing all species isolated from each tissue type and each orchard are available on request from the author.

In kiwifruit several of the fungi isolated from symptomless leaf tissue cause important post-harvest fruit rots in these crops (Pennycook 1985; Pennycook and Samuels 1985), including *Botryosphaeria dothidea*, *Colletotrichum acutatum*,

Fusicoccum luteum, *Glomerella cingulata*, and *Phomopsis* spp. Hawthorne *et al.* (1982) reported a similar range of fungi from necrotic leaf tissue of kiwifruit. This may indicate that, biologically, the fungi isolated from leaf tissue are better regarded as latent invaders than true endophytes. The source of the inoculum for these latent infections is uncertain. The increase in levels of infection with age suggests continuing external invasion of the tissue. However, different parts of the leaves are infected at different levels, indicating that factors other than the simple deposition of fungal spores on exposed leaf surfaces are involved. A similar effect is likely with fruit tissue, the fruit-rotting species found associated with symptomless leaf tissue probably also latently infect young fruit, causing rots later as the fruit matures. However, some of the major kiwifruit fruit rots - *Botrytis cinerea*, *Cryptosporiopsis* sp. and *Sclerotinia sclerotiorum* - were never isolated in this study, suggesting a different mode of attack for these species. Apparent differences in the biology of these two groups of fungi may provide information significant for their control.

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