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## SPATIAL VARIATION OF WOOLLY APPLE APHID (*ERIOSOMA LANIGERUM*, HAUSMANN) IN A GENETICALLY DIVERSE APPLE PLANTING

**Summary:** There is an increasing requirement to breed durable resistances to woolly apple aphid (WAA) into apple cultivars. Genetically diverse apple plantings have been established in New Zealand with one aim to identify new sources of resistance to this pest, and also to allow the computation of parameters of genetic interest. Such computations are hindered by the uneven distribution of the pest in the orchard. The spatial distribution of WAA was investigated using local trend surfaces to examine large scale patterns, and point process analyses to check for the presence of small scale clumping. Large scale patterns in WAA distribution were found which could be attributed to the degree of exposure of the trees, and clumping was also detectable. The experimental design was found to adequately accommodate these spatial patterns. The application of the point process analysis to other ecological situations, and manners in which it could be extended, are discussed.

**Keywords:** Spatial point patterns; block designs; resistance; biodiversity; *Eriosoma lanigerum*; *Malus*.

### Introduction

Woolly apple aphid, WAA (*Eriosoma lanigerum*, Hausmann), is a common cosmopolitan pest of apple and is potentially devastating, particularly to apple industries in the Southern Hemisphere. At the end of last century, the New Zealand apple industry was brought to the brink of ruin by this pest, but with the introduction of the North American cultivar, 'Northern Spy', the industry was saved. It was shown in New Zealand that this cultivar was 'blight proof'. Today, its derivatives M.793 and MM.106 remain the most widely used rootstocks for apple in New Zealand.

The tiny specific endoparasitoid *Aphelinus mali* (Haldeman) was successfully established in New Zealand in the 1920s and by the 1930s was widely accredited with providing successful biological control of WAA. Broad spectrum pesticide use also killed the parasitoid, and growers again became reliant on aphicides. Only with the recent change to insecticides based upon insect growth regulators has *Aphelinus mali* again assumed a useful role in WAA control (Shaw and Walker, 1996; Batchelor *et al.*, 1997). Nevertheless, the development of apple cultivars and rootstocks with genetic resistance to the pest will continue to be an important form of defence.

An important aspect of the New Zealand breeding programme is finding new sources of resistance for application in a breeding strategy based on durable resistance. The importance of this

strategy is demonstrated by the complete breakdown of the 'Northern Spy' resistance, or more correctly, the development of virulence to the resistance gene in 'Spy-capable' WAA, in South Africa (Giliomee, Strydom and Van Zyl, 1968).

The Horticulture and Food Research Institute of New Zealand Ltd. has developed an apple breeding population to establish and maintain biodiversity within the *Malus* germplasm for genetic studies and cultivar development (Noiton and Shelbourne, 1992). It is expected that this population will provide sources of mono- and polygenic resistance to a wide range of pests and diseases, as well as information on their heritability. However, screening and analyses of the data may be compromised by uneven distributions of pests and diseases in the field. For example, in New Zealand, WAA generally produces wingless offspring which disperse at the crawler stage. Thus, infestations are clumped in the initial stages. Once infestation becomes heavy, this clumping is generally thought to disappear. This paper reports on the evaluation of part of the apple breeding population for WAA infestation, and the investigation of the spatial distribution of the aphid.

### Methods

The apple breeding population was established over four years at three sites. For this study, one sub-population (at Havelock North, established in 1993)

consisting of trees from 66 families in their 4th leaf was evaluated for WAA infestation. The study was limited to this sub-population as it was the only one which appeared to have a consistent, heavy infestation pressure throughout the planting block. The number of trees in each family ranged from 1 to 101, with a total of 2136 live seedlings in the sub-population. Most families were derived from open-pollinated seed from a diverse range of apple cultivars (*Malus domestica* Borkh.), as well as crab apples (*Malus* spp.). Five families were the result of controlled crosses between apple cultivars. Initial natural infestation had occurred in the nursery, where trees were planted family by family. After the second year, the trees were transplanted into 11 orchard rows at a planting distance of 3 m x 0.75 m where the epidemic was allowed to develop further. Single-tree plots were planted in randomised incomplete blocks of 20 trees allocated using the Designer software (K. Russell, *pers. comm.*; University of Wollongong, NSW, Australia).

The seedlings were maintained using a minimal insecticide and fungicide programme, which each year was stopped in mid-summer. *Aphelinus mali* had not established itself in the orchard and its presence was negligible. Between 28 April and 2 May 1997, the aerial portion of each seedling was rated for WAA infestation on a 5-point scale as follows:

- 5 = no infestation;
- 4 = light infestation consisting of several small, separate colonies;
- 3 = medium infestation and galling with some colonies starting to coalesce;
- 2 = many colonies coalescing and up to 2 shoots completely infested and galled;
- 1 = heavy infestation and galling on more than 2 shoots.

Large scale spatial patterns in WAA distribution were investigated by fitting local trend surfaces to both the raw data, and the difference between the raw data and the family mean. The latter was to account for differences in WAA resistance/susceptibility among the families. Fits were achieved using locally weighted least-squares regression, loess (Cleveland, 1979; Venables and Ripley, 1994). This determines non-parametric smoothed contours by averaging over quadratic response surfaces fitted at each point by weighted least-squares. The weights are chosen to ensure that local data are the most influential. The technique, which is implemented in much statistical software, is well adapted for exploratory work. Residuals from loess response surfaces were examined to check the suitability of the model.

Finer details of WAA distribution were examined by modelling the location of the heavily

infested trees (i.e., WAA score 1) as a point process. Almost one third of the surviving trees were heavily infested, which provided a good proportion to detect any clumping if it existed.

The point process was examined using Ripley's  $K(t)$  function (Venables and Ripley, 1994). If  $\lambda$  is the number of heavily infested trees per unit area, then  $\lambda K(t)$  is the expected number of heavily infested trees within distance  $t$  of another heavily infested tree.  $\lambda K(t)$  was estimated from the data by taking the average, over all heavily infested trees, of the number of heavily infested trees within distance  $t$ , using Ripley's edge correction (Venables and Ripley, 1994). Edge correction was necessary since the circle centred at a tree less than distance  $t$  from the edge will not lie entirely within the orchard block. Thus each neighbouring heavily infested tree was counted as  $1/p$  trees; where  $p$  is the proportion of the circle centred at the tree of interest, and passing through the neighbour under consideration, which lies within the orchard. For example, if the entire circle lies within the orchard, the neighbouring tree is counted once; if only half the circle lies within the orchard the neighbour is counted twice.

For a completely random Poisson process,  $K(t) = \pi t^2$ , which can be linearised by taking  $\sqrt{K(t)}/\pi$ . Larger values represent clustering and smaller values indicate regularity. Since WAA infestations could only occur on the apple trees, and these were planted at regular intervals, the process we were modelling was not completely random. However, it was still possible to compare the observed distribution of WAA with a random allocation of the pest to the trees using randomised re-sampling. The confidence limits were obtained from 100 random permutations of the heavily infested trees onto any location where there was a living, non-immune tree. For each permutation  $K(t)$  was estimated, and the extreme values provided the limits.

The ability of the blocking in the experimental design to account for the observed spatial patterns, and hence allow the estimation of parameters of genetic interest, was tested. Both the local trend surface and Ripley's  $K(t)$  function were re-computed using the residuals after fitting family and block to the raw data. With Ripley's  $K(t)$  function this presented a problem since the residuals no longer represented a dichotomy between heavily infested or not. As an approximation, the residuals were divided into low (infested) or high such that the number of trees in the first category was the same as the number of heavily infested trees in the raw data. All statistical analyses were undertaken using S-Plus ver. 3.3 (Statistical Sciences, 1995), and Ripley's  $K(t)$  function was computed using S-Plus functions written by Ripley (Venables and Ripley, 1994).

## Results

Of the 2136 seedlings, 551 (25.8%) had no WAA present and 682 (31.9%) were heavily infested. A high proportion of the non-infested trees were at the unsheltered end of the rows (Fig. 1). There was also a tendency for trees in northern rows to be less heavily infested by WAA. The local trend surface fitted an equivalent of 7.1 parameters; the fractional number of parameters reflects the non-parametric nature of the procedure. These parameters represent approximately 0.3% of the total degrees of freedom, and give a multiple  $R^2$  value of 14%. Clearly, the trend surface identified a real overall spatial pattern, but a great deal of variation in infestation level remained unexplained. The pattern was unaltered in its essentials by accounting for differences between WAA susceptibility among the families. The frequency distribution of the residuals was bimodal: the higher mode was attributable to the resistant trees, and the lower mode resulted from the high

frequency of heavily infested trees. As expected, when the local trend surface was fitted to the residuals from fitting a linear model of the raw scores on block and family, the multiple  $R^2$  dropped to negligible (~2%).

Ripley's  $K(t)$  function, estimated using the raw data of all 2136 trees, fell within the confidence interval for distances beyond about 25m (Fig. 2a). However, for distances less than this the estimated function was greater than the upper limit; i.e. there were more heavily infested trees within 20-25 m of a heavily infested tree than would be expected if the distribution were random. The computations were repeated, this time without row 11 and omitting trees at the end of the row (>200), thereby removing much of the observed large scale spatial pattern (Fig. 1). Ripley's  $K(t)$  function was then found to be within the confidence limits for distances over about 8m (Fig. 2b). However, there was strong evidence of clumping at distances less than this.

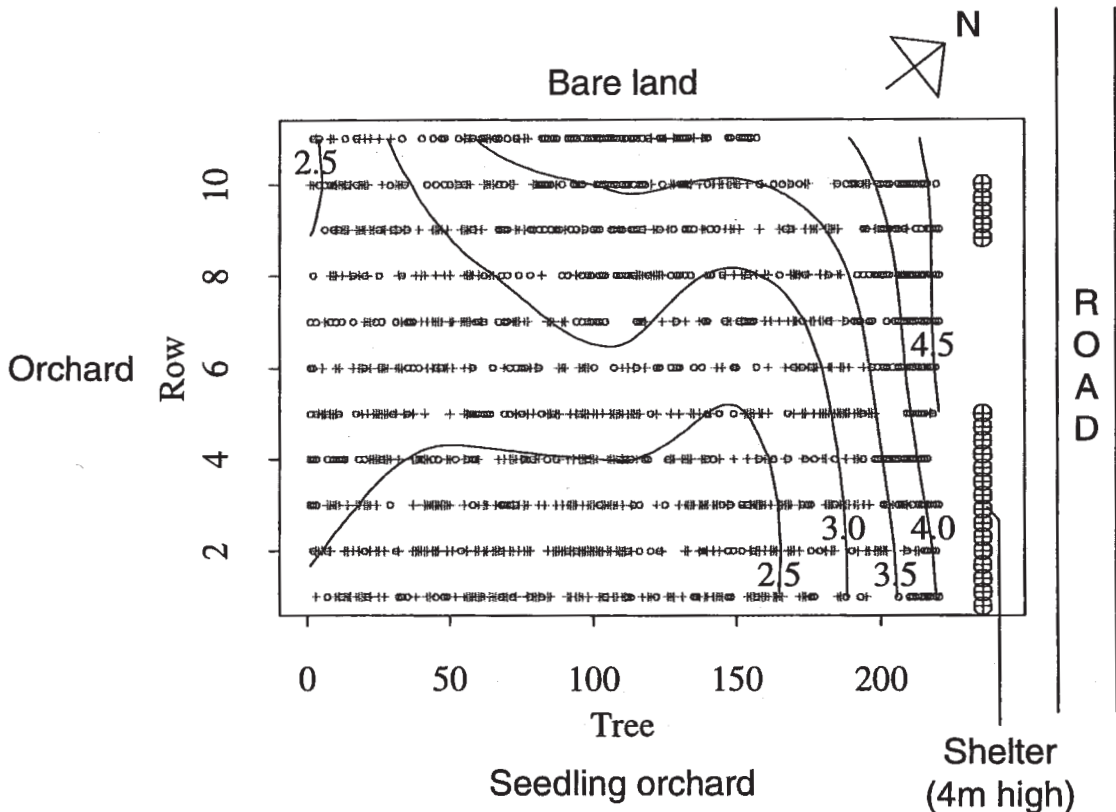


Figure 1: Contour plot showing the fitted local trend surface for the WAA infestation scores, and the location of heavily infested (+) and non-infested (o) trees. Row 11 was incomplete (only 157 trees).

The value of Ripley's  $K(t)$  function computed using the residuals after fitting block and family to the raw scores generally fell within the confidence limits (Figs. 3a and b). When all rows and trees were

used, the function was below the lower limit for distances of 15-25 m. It was near the lower level for distances of 15-18 m when row 11 and the end trees were omitted from the analysis.

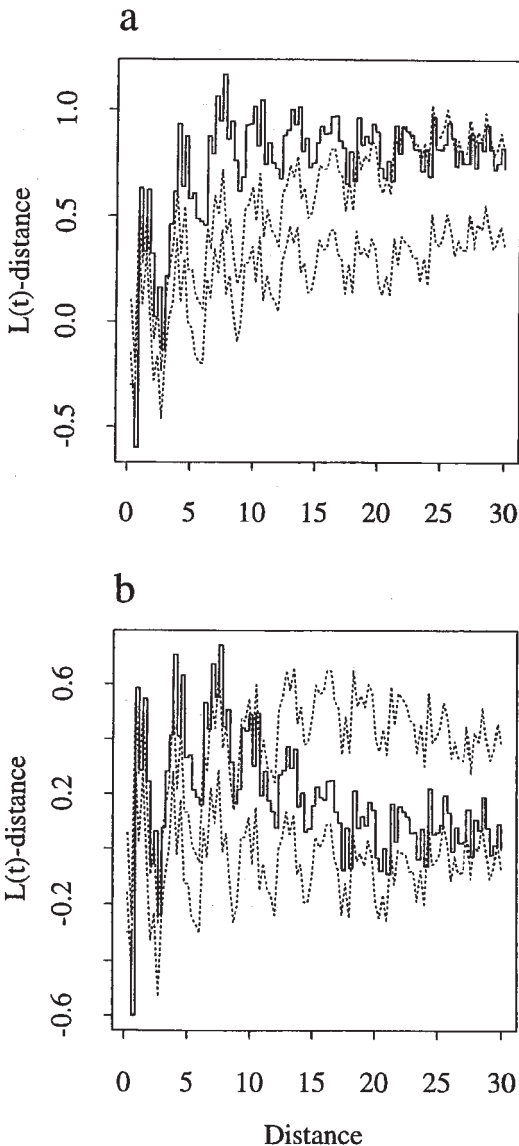


Figure 2: Plots of  $L(t)$ , being a transformation  $\sqrt{K(t)/\pi}$  of Ripley's  $K(t)$  function computed from the point location of the heavily infested trees, less the distance  $t$ , against the distance: (a) using all trees, and (b) excluding row 11 and the last 20 trees in the remaining rows. The confidence limits obtained from 100 random permutations are given by the dotted lines.

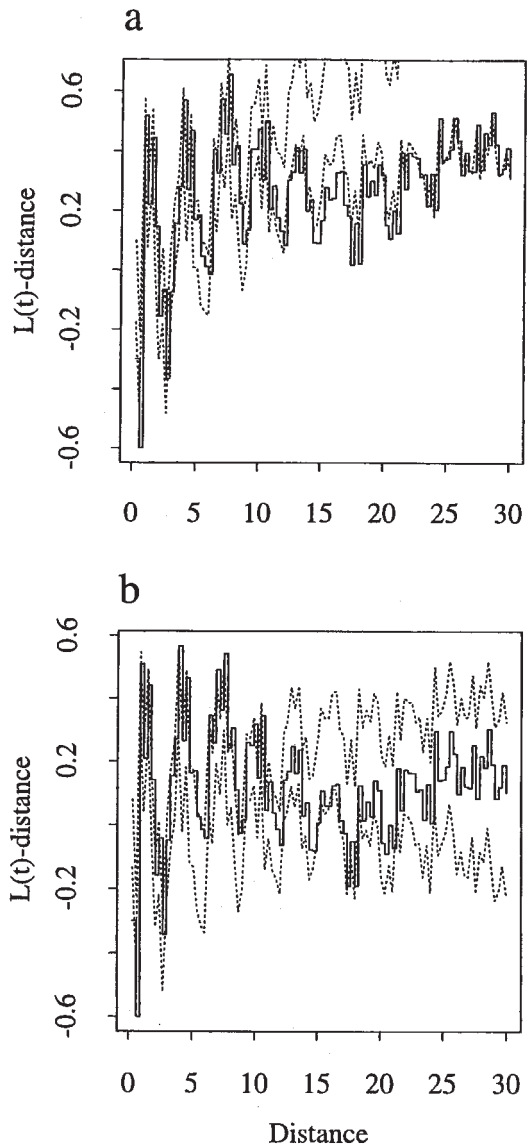


Figure 3: Plots of  $L(t)$ , being a transformation  $\sqrt{K(t)/\pi}$  of Ripley's  $K(t)$  function computed from the residuals after fitting block and family, less the distance  $t$ , against the distance: (a) using all trees, and (b) excluding row 11 and the last 20 trees in the remaining rows. The confidence limits obtained from 100 random permutations are given by the dotted lines.

## Discussion

The randomisation process would have ensured that genetic resistances and the infestation apparent in the nursery were initially randomly distributed in the field. However, woolly apple aphid appeared to be distributed in a non-random manner both on a macro- and micro-level. At the macro-level, the infestation was less in the exposed sites: at the eastern end of the rows and in the northern rows which were less sheltered. The clumped distribution observed at the micro-level may be attributed to the mode of dispersion of the aphid. Even in a heavily infested block like the one under study, this clumped distribution was still detectable. Clumping may be minimised by inoculating all seedlings, which is being done with the 1994 sub-population. It is expected that in this case any variations in the spatial distribution of WAA will be mainly due to variations in environmental conditions, such as different levels of exposure.

The experimental design incorporated blocking to account for global trends in spatial variation. The chosen block size of 20 trees was comparatively small so that relatively fine spatial patterns could be managed. That there was no large scale pattern in the spatial distribution of the residuals after fitting families and blocks was expected as the sum of the residuals for any block would be zero. However, the ability of the blocking to handle the smaller scale pattern attributable to the clumping of the aphids was gratifying. The tendency of Ripley's  $K(t)$  function computed from the residuals (Figure 3) to be near, or under, the lower confidence limit around 15 m (i.e., 20 trees at 0.75 m spacing) reflects spatial uniformity between blocks enforced by fitting blocks.

The analysis of the spatial patterns in WAA distribution suggests that estimates of heritability and general combining ability will not be unduly influenced by spatial variation, provided the blocking structure is included in any such estimation. Had a much larger block size been selected in the design of this experiment, it is likely that some form of near-neighbour analysis would have been necessary to control variation unaccounted for by blocking. Alternatively, the original plantings could have been interspersed with regularly-spaced control clones. However, this entails an extra resource overhead, which our design and subsequent analysis have proven to be unnecessary.

Ripley's  $K(t)$  has a wide application in the investigation of point processes in ecology. Examples include the spatial distribution of animals on some fixed substrate (e.g., lizards on rocky

outcrops, scale insects on trees), and the distribution of plant species which may exhibit the full range from clumped (due to restricted dispersion) to uniform (due to some form of mutual exclusion). The method can be extended to allow for multi-type point patterns (e.g., our full 5-point scale rather than simply heavily infested or not) (Cressie, 1991). Also, in computing the confidence limits, the random permutations can be modified to allow for differences in the probability of infestation, perhaps attributable to tree size or family. Furthermore, alternatives to a random process could be investigated by using the alternative models to compute the confidence interval. However, the method is computational intensive and, given that the investigation into the spatial patterns was of only secondary concern to us, the extra investment did not seem justified. In other circumstances this may not be the case.

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