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COMPARATIVE EPIDEMIOLOGY OF THE PERSISTENTLY TRANSMITTED SCRLV AND THE NON-PERSISTENTLY TRANSMITTED BYMV, AND DEVELOPMENT OF MOLECULAR HYBRIDIZATION ANALYSIS AS A DIAGNOSTIC METHOD FOR SCRLV.

by

Kithsiri Wimal Jayasena B.Sc.(Hon.) Cey.

Department of Plant Pathology,

Waite Agricultural Research Institute,

The University of Adelaide

South Australia

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SUMMARY

Comparisons were made simultaneously of the spread of the non-persistently transmitted bean yellow mosaic virus (BYMV) and the persistently transmitted subterranean clover red leaf virus (SCRLV) in field plots of Vicia faba L. minor and Trifolium subterraneum L. The study was made in a Mediterranean environment.

Spread from a primary source was mapped following the artificial introduction of virus alone, or virus with vector at the centre of the plots. A gradient originated from the source of BYMV placed centrally in the plots and its shape was independent of whether or not the source was colonized with the vector aphid species Aphis craccivora Koch. and Aulacorthum solani (kltb.). By contrast, SCRLV spread from the source only when plants were also artificially infested with the vector A.solani (kltb.). This suggests that the spread of SCRLV is dependent on vector colonization. The gradient of infection observed for BYMV and SCRLV in the control plots where neither virus nor vector were provided centrally indicated that both viruses spread into the plots from an outside source, presumably from the nearby plots.

An attempt was made to evaluate the importance of secondary spread of both viruses by assessing the degree of clumping of infected plants that occurred outside the primary sites of virus introduction. With BYMV, clumping of infected plants occurred whether virus was introduced with its vector or not as well as in control plots. With SCRLV clumping occurred only when the vectors were introduced on virus source

plants at the beginning of the experiment.

The patterns of distribution of both viruses in the plots were examined using ordinary run analysis (Madden et al., 1982). With BYMV, when the virus source was provided either with or without vectors, the distribution of infected plants was nonrandom. This suggests that BYMV was spreading in the experimental plots from plant to plant. The distribution of SCRLV was nonrandom where the virus source was provided centrally with the vector but it was random when the SCRLV source was provided centrally without the vector. Such random distribution indicates that virus was not spreading from plant to plant and is consistent with the virus coming from an outside source.

In T.subterraneum L. plots similar gradients of infection were observed when a virus source was introduced with vectors at the centre. When virus and vector were not introduced at the centre (control plots) the observed gradient was from the outside and only on the sides proximal to the plots where both viruses were provided with vectors. Analysis of the development of clumps of adjacent infected plants according to their time of appearance showed that more clumps appeared in the plots where the virus and vector were provided artificially compared with the control plots, and that peak clumping occurred at the time of peak rate of spread of both viruses.

Time of spread of both viruses was determined by exposing trap plants at 4 weekly intervals throughout the 30 month trial period. Both viruses spread in the spring when vectors were flying, but negligible spread of the viruses was observed in the

autumn despite aphid flight activity.

Times of flight of the four main aphid vector species were continuously monitored with yellow water traps. A major spring and a minor autumn flight peak were observed for Aphis craccivora koch., Macrosiphum euphorbiae (Thomas), Aulacorthum solani (kltb.) and Myzus persicae (Sulz.). Analysis of weather data showed that the aphid flights occurred predominently in weeks when the mean weekly temperature was in the range 13 - 17°C. Weekly rainfall above 7 mm per week appeared to affect the flights only when mean weekly temperatures were outside the range 13 - 17°C.

A replicated trial was done in one crop growing season to determine whether treatment with the insecticides Disyston, Metasystox or Malathion, or a barley barrier row, influenced the spread of BYMV or SCRLV from infected to healthy V. faba L. plants. Their effect on the aphid population in the plots was also determined by sampling V. faba L. plants. The results indicate that although the insecticide treatments reduced the aphid population, they did not affect the pattern of spread of either BYMV or SCRLV. However the barley barrier influenced the pattern of movement of BYMV and not SCRLV while no effect was seen on the aphid population. The overall incidence of SCRLV, in the plots receiving Metasystox and Disyston was less than that obtained with the other treatments but no marked difference was observed between treatments for BYMV.

An electronmicroscopic study was made of T.subterraneum L.cv Mt.Barker infected with SCRLV. In thin sections obtained from

phloem of both leaves and stems, small isometric virus-like particles were detected in the transfer cells, but not in xylem or mesophyll cells. Healthy T.subterraneum L. contained no virus-like particles. Virus-like particles similar in size (30.4 nm diameter) and appearance but serologically distinct from those of potato leaf roll virus were also detected in purified preparations from SCRLV infected T.subterraneum L. plants.

Subterraneum clover red leaf virus (SCRLV) was purified from infected Trifolium subterraneum L. plants or Pisum sativum L. by either of two methods, both of which employed cellulase digestion of crude extracts. The PEG (polyethylene glycol) method using infected P.sativum L. plants gave a higher yield of virus (1.34 mg/kg) than the other method (24 µg/kg) where PEG was not used. Only traces of virus were recovered from tissue of infected T.subterraneum L. These virus particles had a bouyant density of 1.31 g/cm^3. in Cs_2SO_4 and A_{260}/A_{280} ratio of 1.5. The relationship of this isolate to the New Zealand isolate of SCRLV was studied with gel diffusion tests and showed a reaction with N.Z. antiserum. In immunosorbent electron microscopy tests, large numbers of particles of the SCRLV Tasmanian isolate became attached to grids coated with antisera prepared to the New Zealand isolate of SCRLV.

Polyacrylamide-urea gel electrophoresis of disrupted virus particles revealed two RNA species and one DNA species. The slow moving RNA and the fast moving RNA had estimated molecular weights of 2.08×10^6 and 1.08×10^6 respectively in these denaturing gels.

H^3 - labelled complementary DNA (cDNA) reverse transcribed from high molecular weight RNA of purified virus was specific for the detection of SCRLV, in that it showed no hybridization with nucleic acids from either healthy plants (T.subterraneum L., Pisum sativum L.) or plants (Physalis floridana Rydb.) infected with the serologically related potato leaf roll virus (PLRV) or nonviruliferous aphid vector Aulacorthum solani (kltb.) but hybridized with homologous RNA and nucleic acids from SCRLV infected plants of two species (T.subterraneum L., P.sativum L.) and viruliferous aphid vector A.solani (kltb.). The cDNA detected SCRLV in individuals and groups of the A.solani (kltb.) and the average virus content was greater than 157 pg per aphid. The enzyme linked immunosorbent assay (ELISA) confirmed the results of molecular hybridization analysis (MHA).