Our studies revealed that the 16S rRNA gene of CGD bacterium is highly conserved and can be used for quick PCR-based detection of CGD bacterium of Nagpur orange plant for the phytosanitary and certification programme. This study provides molecular evidence for the association of *Ca.* Liberibacter asiaticus with Nagpur orange decline in the Vidarbha region. For requirement of certified planting material, citrus growers of this region use locally produced non-certified planting material by private nurseries. Mother plants from which buds were taken for large-scale propagation were often infected with CGD bacterium, which resulted in the spread of the disease on a wider scale rendering the plantation with poor yield potential. Moreover, presence of psyllid vector made the situation even worse. Under these circumstances, it is vital to ensure that growers have access to CGD-free planting material for establishing a profitable citrus industry in this part of India.

Nucleotide sequence accession number. The representative DNA nucleotide sequence of *Ca.* Liberibacter asiaticus Nagpur isolate has been deposited in the GenBank database under accsession no. EU 939452.

1. Naqvi, S. A. M. H., In *Diseases of Fruits and Vegetables*, Kluwer, The Netherlands, 2004, vol. 1, pp. 247–290.
2. Ahlawat, Y. S., *Indian J. Agric. Sci.*, 1997, **67**, 51–57.
3. Bové, J. M., *J. Plant Pathol.*, 2006, **88**, 7–37.
4. Jagoueix, S., Bové, J. M. and Garnier, M., *Int. J. Syst. Bacteriol.*, 1994, **44**, 379–386.
5. Capoor, S. P., Rao, D. G. and Viswanath, S. M., *Indian J. Agric. Sci.*, 1967, **37**, 572–576.
6. McClean, A. P. D. and Oberholzer, P. C. J., *S. Afr. J. Agric.*, 1965, **8**, 297–298.
7. Hocquellet, A., Toorawa, P., Bove, J. M. and Garnier, M., *Mol. Cell. Probes*, 1999, **13**, 373–379.
8. Das, A. K., Rao, C. N. and Shyam Singh, *Curr. Sci.*, 2007, **92**, 1759–1763.
9. Jagoueix, S., Bové, J. M. and Garnier, M., *Mol. Cell. Probes*, 1996, **10**, 43–50.
10. Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D. J., *Nucleic Acids Res.*, 1997, **25**, 3389–3402.
11. Thompson, J. D., Higgins, D. G. and Gibson, T. J., *Nucleic Acids Res.*, 1994, **22**, 4673–4680.
12. Hall, T. A., *Nucleic Acids Symp. Ser.*, 1999, **41**, 95–98.
13. Tamura, K., Dudley, J., Nei, M. and Kumar, S., *Mol. Biol. Evol.*, 2007, **24**, 1596–1599.
14. Saitou, N. and Nei, M., *Mol. Biol. Evol.*, 1987, **4**, 406–425.
15. Felsenstein, J., *Evolution*, 1985, **39**, 783–791.
16. Takezaki, N., Rzhetsky, A. and Nei, M., *Mol. Biol. Evol.*, 2004, **12**, 823–833.
17. Tamura, K., Nei, M. and Kumar, S., *Proc. Natl. Acad. Sci. USA*, 2004, **101**, 11030–11035.

Received 1 September 2008; accepted 13 February 2009

A. K. DAS

National Research Centre for Citrus,
Amravati Road,
Nagpur 440 010, India
e-mail: dasashiskumar@hotmail.com

Gut content analysis of spiders in coffee ecosystem

Spiders are gaining favour in ecological studies as indicators of environmental quality[1] and as biological control agents in agricultural ecosystems[2]. They are the abundant natural enemies in any agro-ecosystem and are found in most terrestrial habitats, often present in large numbers[3]. All spiders are predaceous and insects constitute their primary prey[4]. They are generalist predators that may be of importance in reducing and even preventing outbreaks of insect pests in agriculture[5]. Spiders tend to be small, cryptic feeders, have extra-oral digestion and sucking mouthparts, and exhibit amorphous gut contents. All these attributes make it difficult to obtain data on predation rates[6]. Some information can be gathered by direct observation[7], but gut analysis of field-collected spiders is the least disruptive and the most efficient means to acquire data on predation[6].

Coffee, a major commercial crop of India harbours rich spider diversity[8]. The crop is also attacked by a variety of pests, including, the coffee green scale, *Coccus viridis* (Green), a major sucking pest of coffee. This correspondence discusses the importance of gut content analysis of spiders in coffee ecosystem and provides suggestions to overcome the difficulties faced in the present techniques. The analysis was carried out with a hypothetical assumption that *C. viridis* is a prey for foliage-dwelling spiders, and *Aularches* sp. could be the prey of spiders inhabiting field margins. This study will lead to better assessment of the predation of *C. viridis* by spiders and their biocontrol potential in coffee plantations. Observations on the analysis of the prey remnants in the gut of spiders are discussed.

Gut content analysis of freshly collected spiders from the coffee plantations of Horticultural Research Station, Yercaud, Tamil Nadu, was carried out by electrophoresis, which is based on the detection of prey enzymes in homogenates of the predator after PAGE and staining for esterase activity[9,10] to know their preferential feeding habit under field conditions. Two different preys (*C. viridis* and an acridid, *Aularches* sp.) and eight species of spiders (*Leucauge decorata* (Blackwall), *Oxyopes* sp., *Dieta virens* (Thorell), *Olios milleti* Pocock, *Telomania dimidata* (Simon), *Clubionia* sp., *Hippasa* sp. and *Plexipus* sp.) were used in the experiment. Extraction was carried out according to the method described by Amalin *et al.*[11], with slight modifications. The samples were macerated in phosphate buffer at the rate of 1 ml/g of the substrate. Electrophoretic separation of isozymes was achieved with 10% native PAGE, as described by Laemmli[12], with slight modifications as required. Polyacrylamide slab gels with a total gradient concentration of 10% and a cross-link gradient of 4% were prepared between two glass plates using a gel gradient maker. Casting of the gel was done similar to that of SDS–PAGE. The whole electrophoresis set-up was kept in the refrigerator for cooling during the run. Sample solutions diluted with sample buffer were loaded onto the sample wells carefully with the help of a microsyringe. The run was continued until the dye front reached the bottom of the gel. After elec-

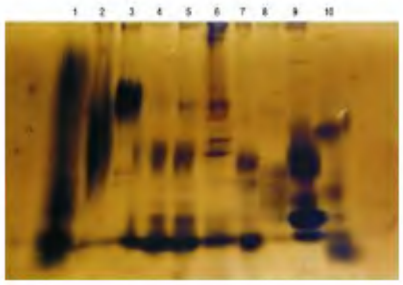

Figure 1. Esterase patterns of prey and spiders. Lanes 1 and 2, *Coccurs viridis* and an acridid, *Aularches* sp.; lanes 3 to 10, *Leucauge decorata*, *Oxyopes* sp., *Dieta virens*, *Olios milleti*, *Telomania dimidiata*, *Clubiona* sp., *Hippasa* sp. and *Plexipus* sp. respectively.

trophoresis, the gel was stained (sodium dihydrogen phosphate, 2.8 g; disodium hydrogen phosphate, 1.1 g; Fast blue RR salt, 0.2 g; alpha naphthyl acetate, 0.03 g and water, 200 ml) for visualization of esterase bands. The gel separated from the glass plate was submerged in the medium for 20–30 min, kept inside a dark container and shaken on a shaker at a speed of 28 rpm for 24 h or until the bands appeared. The reaction was stopped by adding a mixture of methanol : water : acetic acid : ethanol in the ratio of 10 : 10 : 2 : 1. Conclusions about the identity of prey remnants inside the gut of the predators were drawn by visual comparison of the esterase patterns[13].

Predation remains one of the most difficult ecological processes to study, but is critical to understand if we have to use predators more effectively in agricultural pest control[14]. The most commonly used approach has been to analyse the extracts from homogenized predators (or just their guts) on polyacrylamide gels and stain for enzyme activity, especially esterases[15,16]. Esterase was selected as the indicator protein because its detection employs an enzymatic reaction with the substrate yielding a stain with the high extinction coefficient[9]. Specific esterase activity pattern allows the identification of prey remnants inside the predator gut[10].

In the present study, esterase bands similar to that of *C. viridis* were noticed with the foliage dwelling spiders, *Oxyopes* sp., *D. virens*, *O. milleti* and *T. dimidiata* (Figure 1), confirming the predation of *C. viridis* by them. A weak esterase band similar to that of *C. viridis* was observed with *Clubiona* sp., which indicates that the number of individuals consumed by the spider was less or the prey would have been partially digested in the gut. This difference in intensity could possibly be used to quantify the number of prey consumed by the predator[11]. Esterase banding pattern of *L. decorata*, *Hippasa* sp. and *Plexipus* sp. was similar to that of the acridid, revealing greater possibility of the acridid as one of the preys for spiders in field margins and strips. The intensity also varied, indicating a possibility of digestion of the prey or degradation of the bands. The banding pattern was more complex and there was no species-specific separation of the bands. This might be due to the presence of other prey in the gut of the spiders, which can be overcome by analysing all the suspected prey existing in the field. Spiders in the field generally feed on multiple prey species. Moreover, not all esterases of a prey may be found in the gut of the spiders. Certain esterases may be localized in tissues that are not ingested, complicating the identification of the prey for field-collected specimens. Therefore, it is necessary to co-electrophorese all suspected prey species with the spiders of the same gel[11], if we want know the prey range of the spiders. Electrophoresis is more effective when the predator feeds on a restricted prey range[17,18]. However, this is not possible under field conditions. Hence, further studies to know the prey preference of field spiders by gut content analysis should be carried out with improved molecular tools for accurate determination of the diet of the spiders.

1. Maelfait, J. P., Jocque, R., Baert, L. and Desender, K., *Acta Zool. Fenn.*, 1990, **190**, 261–266.
2. Bishop, L. and Riechert, S. E., *Environ. Entomol.*, 1990, **19**, 1738–1745.
3. Kaston, B. J., *How to Know the Spiders*, M.C. Brown, Iowa, 1978, 3rd edn, p. 272.
4. Turnbull, A. L., *Annu. Rev. Entomol.*, 1973, **18**, 305–348.
5. Sunderland, K. D., Fraser, A. M. and Dixon, A. F. G., *J. Appl. Ecol.*, 1986, **23**, 433–447.
6. Stuart, M. K. and Greenstone, M. H., *Ann. Entomol. Soc. Am.*, 1990, **83**, 1101–1107.
7. Greenstone, M. H., *J. Arachnol.*, 1999, **27**, 333–342.
8. Senthil Kumar, C. M. and Regupathy, A., In 16th International Congress of Arachnology, Belgium, 2–7 August 2004, p. 150.
9. Murray, R. A. and Solomon, M. G., *Ann. Appl. Biol.*, 1978, **90**, 7–10.
10. van der Geest, L. P. S. and Overmeer, W. P. J., *Meeded. Fac. Landbouww. Rijksuniv. Gent.*, 1985, **50**, 469–471.
11. Amalin, M. D., Pena, J. E. and McSorley, R., *Fla. Entomol.*, 2000, **83**, 489–492.
12. Laemmli, U. K., *Nature*, 1970, **227**, 680–685.
13. Sadasivam, S. and Manickam, A., *Biochemical Methods*, New Age International (P) Ltd, New Delhi, 1996, p. 230.
14. Naranjo, S. E. and Hagler, J. R., *Biol. Control*, 2001, **20**, 175–189.
15. Schelvis, J. and Siepel, H., *Entomol. Gen.*, 1988, **13**, 61–66.
16. Walrant, A. and Loreau, M., *Entomol. Gen.*, 1995, **19**, 253–259.
17. Dicke, M. and De Jong, M., *Exp. Appl. Acarol.*, 1988, **4**, 15–25.
18. Solomon, M. G., Fitzgerald, J. D. and Murray, R. A., In *The Ecology of Agricultural Pests: Biochemical Approaches* (eds Symondson, W. O. C. and Liddell, J. E.). Chapman and Hall, London, 1996, pp. 457–468.

ACKNOWLEDGEMENTS. C.M.S.K. thank Dr M. Ganesh Kumar, FC and RI, Mettupalayam and Dr Manju Siliwal, Zoo Outreach, for help in the identification of spiders. Thanks are also due to Dr Djanagiraman for help while conducting the experiments.

Received 22 April 2008; revised accepted 19 February 2009

C. M. SENTHIL KUMAR[1,2,*]
A. REGUPATHY[1]

[1]*Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore 641 003, India*
[2]*Present address: North East Institute of Science and Technology (CSIR), Substation, Imphal 795 004, India*
**For correspondence.*
e-mail: cmskm@yahoo.com