Progress With Greening Research in South Africa

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ABSTRACT. Since the early 1950's, greening research in South Africa has concentrated mainly on the isolation and identification of the African greening organism (GO). Various bacterial taxa were isolated from greening-infected and symptomless citrus material (1), confirming the report by Gardner, Feldman and Zablotowicz (2) that bacteria occur naturally in citrus tissue. Monoclonal antibodies (MA) were produced against one of the isolates suspected of being involved in the greening syndrome, viz. *Acinetobacter lwoffii*. However, these MA did not react consistently with greening-diseased plant material or with the greening vector (*Trioza erytreae*) (7). Nevertheless, 13% of 46 Asian greening-infected citrus samples from the Philippines gave a strong positive reaction with the *A. lwoffii* MA, thus suggesting that the organism could play an ancillary role in citrus greening.

Research efforts in South Africa are now directed at producing MA against the African GO *in situ*. MA were raised against African greening according to the indirect approach described by Garnier *et al.* (3) for Asian greening, using greening-infected psylla as immunogen. This was done since the GO can multiply to high numbers in psylla gut, haemolymph and salivary glands before being transmitted (6). Greening positive and negative psylla colonies were established and maintained in insect-proof cages in the greenhouse. The positive or negative nature of the psylla sources were confirmed by vector transmission and subsequent observation of symptoms as well as transmission electron microscopy. The immunisation procedure of Lin and Chen (5) was followed, and cell fusion was carried out according to the technique of Köhler and Milstein (4). An indirect ELISA (7) was optimized to screen supernatants.

Eight of 128 wells containing hybridomas gave a positive ELISA reaction with greening-positive psylla. In a second screening of the hybridomas, columella from symptomatic greening-positive citrus yielded 6 of 158 ELISA-positive wells. The four hybridomas with the highest ELISA signal to background ratio (R) (Table 1) were tested further against greening negative psylla and columella. Two cell lines were identified as potential pathogen binders due to their high R values, as indicated by a strong signal with greening-infected psylla.

TABLE 1
SUMMARY OF ELISA EVALUATIONS OF SELECTED CELL LINES SCREENED WITH GREENING-INFECTED AND HEALTHY COLUMELLA AND PSYLLA EXTRACTS

Cell line code	Antigen	R+	R–	R¹	Interpretation
3G6	Columella	2.18	2.55	0.85	Negative binder
3G6	Psylla	2.19	2.87	0.76	Negative binder
3F3	Columella	1.74	1.75	0.99	Negative binder
3F3	Psylla	2.69	3.79	0.70	Negative binder
1H12	Psylla	2.86	1.19	2.40	Potential pathogen binder
2H3	Psylla	3.22	1.76	1.88	Potential pathogen binder

R = signal over background (antigen absorbance (ABS) reading/ DMEM-HS abs); R+ = antigen positive for greening (greening infected material); R– = antigen negative for greening (healthy citrus material); R¹ = R+/R–; DMEM-HS = background.

Cloning and subcloning of promising hybridomas have been completed and further evaluation is in progress. Greening positive and negative psylla and phloem tissue will be collected from different citrus-producing areas in South Africa to determine the specificity of the clones.

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