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A comparative study in five laboratories on detection of *Clavibacter michiganensis* subsp. *sepedonicus* in potato stems and tubers

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Summary

Potato stems and tubers grown in the field from seed tubers inoculated with *Clavibacter michiganensis* subsp. *sepedonicus*, which causes bacterial ring rot, were tested by indirect, sandwich enzyme-linked immunosorbent assay (ELISA) in five laboratories. Correlation between values for each experimental treatment from the five laboratories was greater ($r = 0.86$) than correlation between values for individual samples ($r = 0.71$). When three or more laboratories obtained ELISA values of ≥ 0.200 for a sample, that sample was presumed to be positive. Conversely, when three or more laboratories obtained ELISA values < 0.200, the consensus determination was regarded as negative. The percentage of stem and tuber samples that were in agreement with the consensus ELISA determination varied from 65.5 to 96.7%. Indirect immunofluorescence tests, conducted on the same samples in two laboratories, were consistent with 83.4 – 91.9% of the consensus ELISA determinations. Presence or absence of *C.m. sepedonicus* was confirmed in some samples by an eggplant bioassay and direct isolation of the bacterium. The ELISA procedure was well suited for screening large numbers of samples and this study confirms it to be a promising procedure in routine indexing of seed potatoes for *C.m. sepedonicus*.

Introduction

Economic, climatic, and marketing factors justify movement of plant material among diverse geographic regions despite the concomitant danger of introducing plant pathogens to new regions. Most countries have developed quarantine regulations to avoid, or minimize, the chance of importing pathogens that are potentially detrimental to national agricultural production. The risk of inadvertent spread of hazardous pathogens in plant material can also be minimized by the use of labora-

tory tests to detect them; such tests must be robust, accurate, sensitive, and reproducible.

Serological tests are becoming increasingly useful for detecting plant pathogenic bacteria in seed and other plant propagules (Saettler et al., 1989). Enzyme-linked immunosorbent assay (ELISA) and immunofluorescence microscopy have often been used and have potential for detecting the bacterial ring rot pathogen, *Clavibacter michiganensis* subsp. *sepedonicus* (Spieck. & Kotth.) Davis et al. (syn. *Corynebacterium sepedonicum* (Spieck. & Kotth.) Skapt. & Burkh.), in potato when symptoms are obscure or absent (Samson & Poutier, 1979; Corbière et al., 1987; Zielke & Kalinina, 1988; De Boer & McCann, 1990). Also eggplant bioassay and direct isolation alone or in combination with serology have been used for ring rot detection and diagnosis (Anon., 1987).

This project was initiated to determine the efficacy of ELISA for detecting the bacterial ring rot pathogen in potato. Data from five laboratories for subsamples from the same field grown potato stems and tubers were analyzed and compared. Some results were verified by immunofluorescence, eggplant tests, and direct isolation. Differences in detection levels for the three cultivars and three inoculation levels used in the experiment are discussed elsewhere (De Boer et al., 1992).

Materials and methods

Field experiment. Cut seed potato tubers of cultivars Red Pontiac, Russet Burbank and Désirée were vacuum inoculated with strain R8 of *C.m. sepedonicus*, at approximately 10^2, 10^4, and 10^6 cfu/ml (Log 2, Log 4, and Log 6 inoculum levels, respectively) and with sterile Ringer's solution ("check" treatment). The seed pieces were planted in field plots as described previously (De Boer et al., 1992) and four stems were collected from each plot on seven dates at two-week intervals. Four tubers from each plot were tested 26 and 56 days after harvest.

Tissue samples. For each stem sample, adhering soil was washed off with running tap water and a 6 cm segment spanning the soil level was cut into 5 – 7 smaller pieces with clean pruning shears and crushed in a plastic bag with 3 ml of distilled water. The liquid was recovered and the volume adjusted to 6 ml with distilled water. Lugol's iodine was added to each sample at 10 μl/ml as preservative. Samples were distributed into 1 ml aliquots in micro-tubes (Bio-Rad, Richmond, CA 94804), sealed and sent via courier to cooperating laboratories in Vancouver, British Columbia, Canada; Fredericton, New Brunswick, Canada; Harpenden, England; Wageningen, the Netherlands; and Merelbeke, Belgium (laboratories are arbitrarily designated Lab A-E in the text).

Unprocessed stem sections (10 cm long) adjacent to sections used for the samples described above from one block each were sent to Labs B and C. Samples usually arrived in good condition at all locations within 48 – 72 h.

Tubers were sampled by removing a core of tissue (ca 1 g) from the stolon end with the tip of a potato peeler and prepared for testing and shipment in the same way as the stems.

ELISA tests. The same protocol was used in all laboratories for ELISA to test samples in duplicate immediately upon receipt. In Lab C samples were stored at -20

°C until all of them had been received and were then tested without duplication.

Ninety-six-well polystyrene Linbro plates (Flow Laboratories, McLean, VA 22102) were coated overnight with the immunoglobulin fraction from a polyclonal anti-*C.m. sepedonicus* antiserum at 5 μg/ml. After washing, 50 μl of sample buffer (2% polyvinylpyrrolidone, 0.2% skim milk powder, and 0.05% Tween 20 in 0.01 M phosphate buffered saline (PBS), pH 7.2) was placed in each well before adding 50 μl of sample. This mixture was incubated overnight in the wells at 4 °C. Plates were washed and blocked with 0.2% skim milk powder in PBS for 30 min at 37 °C and then sequentially incubated with monoclonal 1H3, anti-mouse antibody conjugated to alkaline phosphatase (Jackson Immunoresearch Laboratories, Inc, West Grove, PA 19390), and p-nitrophenyl substrate as described by De Boer et al. (1988). All incubations were for 1 h at 37 °C. Absorbance was recorded at 405 nm with Titertek (Flow Laboratories) plate readers.

Positive and negative control samples were prepared by one laboratory and shipped to all cooperating laboratories. Positive controls for the ELISA test consisted of a pure culture of *C.m. sepedonicus* strain R8 adjusted to $OD_{660} = 0.5$. Negative controls consisted of healthy, greenhouse grown potato stems treated the same way as the samples. Four positive and four negative control wells were included on every ELISA plate. ELISA data from each laboratory was normalized separately by the following transformation: $X_t = (X_m - Y_n)/(Y_p - Y_n)$, where X_t and X_m represented the transformed and actual mean of the duplicate ELISA readings respectively, and Y_n and Y_p represented the mean of negative and positive control readings, respectively. Transformed values were used for data analysis.

ELISA determinations were regarded positive if the transformed value was ≥ 0.200. The consensus determination was considered positive only if three or more of the laboratories obtained a positive ELISA reading. If three or more laboratories obtained a negative ELISA reading the consensus determination for that sample was considered negative.

Immunofluorescence. Labs B, D, and E conducted immunofluorescence tests on samples prepared for ELISA. Lab B did immunofluorescence only on samples that corresponded to stems used for the eggplant bioassay. At Lab B samples received for the ELISA test were diluted 10-fold to 1:1000, applied in 25 μl volumes to wells of multi-well microscope slides, and heat fixed. Slides were stored at -20 °C until they were stained by the indirect immunofluorescence procedure using monoclonal antibody 9A1. The mean number of fluorescing cells with typical coryneform morphology in 20 fields selected from two diameters of the well at 630× were recorded.

The quantitative immunofluorescence procedure (De Boer & Hall, 1988) was used by Lab D to estimate *C.m. sepedonicus* population densities in all stem samples. Three decimal dilutions were made of each sample and these together with the undiluted sample were placed on multi-well slides at 20 μl/well. Samples were stained by the indirect procedure using monoclonal antibody 9A1. Bacterial cell numbers were estimated as immunofluorescing units (IFU)/ml of sample.

In Lab E, duplicate 15 μl aliquots of each sample were heat-fixed to separate wells of multi-well slides. Preparations were stained with anti-*C.m. sepedonicus* polyclonal antiserum at 1:3200 and 1:6400 in an indirect immunofluorescence procedure. Stained preparations were evaluated at 500×. The number of fluorescing cells with coryneform morphologies per microscope field was estimated by scanning two

diameters of each well.

Lab C carried out immunofluorescence tests only on isolated vascular bundles dissected from the separate stem segments. Four decimal dilutions of samples (20 μl) were heat-fixed to multi-well slides and stained by the indirect procedure using a polyclonal antiserum. Preparations were evaluated microscopically at 500 × by scanning two diameters at right angles per well. Presence of fluorescent coryneform-shaped cells was considered positive.

Eggplant bioassay and isolation. At Lab B unprocessed stem samples were inoculated into greenhouse-grown eggplants at the two-leaf stage using the procedure described by Lelliott and Sellar (1976). Six plants were inoculated for each sample tested, and eggplants were regularly observed for symptoms over a 40 day period. Symptomatic plants were considered positive for the presence of *C.m. sepedonicus* when microscope slide preparations from them contained many Gram-positive coryneform cells.

The unprocessed stem samples received by Lab C were washed in tap water, dried, and surface disinfected by wiping with 70% ethanol. The lowest 1 cm was discarded and vascular bundles were dissected out over a 2 – 3 cm distance with a sterile scalpel. Separated bundles were macerated in 1 ml 0.05 M PBS with a mortar and pestle and serially diluted 10-fold to 10^{-4}. Three replicates of each dilution were plated at 50 μl/sample onto YGM medium supplemented with 30 mg nalidixic acid, 10 mg polymyxin, and 200 mg cycloheximide per litre. Plates were incubated at room temperature (20 – 25 °C) for up to 21 days. *C.m. sepedonicus*-like colonies were subcultured and identified by slide agglutination using heat-treated cells and polyclonal antiserum.

Results and discussion

Although the same brand of 96-well plates and antibody reagents were used in all participating laboratories, results were not always consistent. There may have been differences in the ELISA plate washing procedure, in buffers due to water quality, and perhaps in storage conditions of the 96-well plates and antibody reagents. Positive and negative control readings were used to measure signal to noise ratio for each laboratory (Table 1). Since good ELISA tests have a high ratio, the test was optimum in Lab E, which had the highest absorbance for positive and lowest absorbance for negative control samples. Lab A and C had the lowest ratios; Lab A had the lowest absorbance value for the positive control and Lab C had the highest absorbance value for the negative control.

Transformation of ELISA values on the basis of the positive and negative control readings standardized the data by minimizing differences due to variation among sampling dates and laboratories. Mean data for each inoculum level/cultivar/sampling date were very similar among Labs B, C, D, and E ($r = 0.87 - 0.94$) (Table 2). Lab A results ($r = 0.72 - 0.83$) tended to vary the most from other laboratories. As expected, correlation of ELISA data for individual potato stems was lower than correlations among mean values (Table 2). Correlation of ELISA values for individual stems was greatest between Labs B and D ($r = 0.88$) and poorest between Labs A and E ($r = 0.52$). The coefficients from duplicate tests within individual laboratories were high (0.99, 0.97, 0.96, and 0.86 for Labs D, E, B, and A, respectively).

Table 1. Mean absorbance values of positive and negative control samples used on ELISA plates for testing potato stems.

Laboratory	Mean absorbance value		
	Positive control	Negative control	Ratio
A	0.693a	0.125c	5.5:1
B	0.826ab	0.092bc	9.0:1
C	1.045ab	0.249d	4.2:1
D	1.106b	0.058a	19.1:1
E	1.583c	0.043ab	36.8:1
S.E.	0.099	0.011	

Values within a column followed by the same letter are not significantly different at $P<0.05$.

Table 2. Correlation between mean absorbance values obtained in ELISA by five laboratories for potato stems sampled at two-week intervals from three cultivars during the growing season.

Lab	Correlation coefficient				
	A	B	C	D	E
A	-	0.60	0.62	0.63	0.52
B	0.75	-	0.82	0.88	0.77
C	0.83	0.94	-	0.84	0.72
D	0.80	0.94	0.94	-	0.72
E	0.72	0.87	0.91	0.89	-

Values in upper right triangle are correlations based on absorbance of 901 individual stem samples for each laboratory; values in lower left triangle are correlations based on mean absorbance of twelve stems for each treatment/cultivar/sampling date for each laboratory.

Although the high level of reproducibility of the ELISA test within laboratories was not attained between laboratories, conclusions on treatment effect were comparable.

In only 54% of all stem samples did all five laboratories agree completely on whether the sample was positive or negative. With the remaining 46% of the samples, at least one of the laboratories had a different result from the consensus determination. For stem samples, results from Lab D agreed most consistently with the consensus determination, whereas Lab C deviated most frequently (Table 3), perhaps because the samples were stored. For tuber samples Lab E differed most frequently from the consensus reaction while Lab A was most consistent (Table 3).

The percentage of stem samples in each laboratory that were positive when the consensus was negative differed significantly between laboratories (Table 4). Lab D had the lowest and Lab C the highest number of false positive reactions. However, the ratio of false positive and false negative reactions was a function of the threshold chosen to delineate positive from negative samples. Therefore it might have been possible to decrease the number of apparent false readings by choosing different threshold values for each laboratory. Nevertheless, the frequency of presumed false

Table 3. Percentage of stem and tuber samples from three cultivars agreeing with the consensus ELISA determination for bacterial ring rot in each laboratory.

	Lab	Cultivar		
		Red Pontiac	Russet Burbank	Désirée
Stems	A	84.5	82.7	79.5
	B	93.5	87.7	91.0
	C	76.8	78.3	84.7
	D	96.7	96.0	93.4
	E	92.9	89.9	90.3
Tubers	A	92.7	88.1	93.2
	B	71.9	86.9	85.2
	C	81.3	65.5	76.1
	D	93.8	88.1	93.2
	E	67.7	91.7	67.0

Table 4. The percentage of stem and tuber samples that were positive by ELISA when the consensus was negative (presumed false positives), and negative by ELISA when the consensus was positive (presumed false negatives).

Lab	Stems [a]		Tubers [b]	
	False positives	False negatives	False positives	False negatives
A	13.98	3.66	6.34	2.61
B	5.99	3.11	1.87	17.16
C	17.75	2.22	24.62	0.75
D	0.44	4.11	4.85	3.36
E	4.88	4.00	20.90	4.10

[a] Stem data based on readings from 901 individual stems.
[b] Tuber data based on readings from 268 individual tubers.

negative reactions (2 – 4%) was fairly constant among laboratories, which confirmed that the chosen threshold was appropriate.

Percentages of presumed false negative and false positive ratings for tubers were also variable among the laboratories (Table 4). The greater number of false reactions compared to stems could be due to a higher and more diversified population of saprophytic bacteria on the tubers. Some bacteria may cross-react serologically or exhibit phosphatase activity that interferes with the ELISA test (De Boer et al., 1988).

The immunofluorescence test has the advantage over ELISA and other serological procedures in that bacterial cells can be seen and their size and shape provide additional information to serological specificity. Moreover, a low epitope copy number and/or affinity of non-target bacteria may be perceived as weak, non-

Table 5. Percentage of samples in which immunofluorescence (IF) results agreed between two laboratories and the consensus ELISA determination.

	Cultivar		
	Red Pontiac	Russet Burbank	Désirée
Agreement in IF between Labs D and E	84.5	81.2	87.2
Agreement between Lab D IF and consensus ELISA	91.9	91.3	89.6
Agreement between Lab E IF and consensus ELISA	88.7	83.4	91.3

Table 6. Isolation of *C.m. sepedonicus* from stems of three potato cultivars grown from seed tubers inoculated at two levels and sampled at four different times; number of stems (out of four) where isolation succeeded.

Days after planting	Red Pontiac		Russet Burbank		Désirée	
	Log 4	Log 6	Log 4	Log 6	Log 4	Log 6
69	0	2	0	0	0	0
83	0	2	0	0	0	0
99	2	2	0	1	0	2
111	2	-[a]	0	1	1	2

[a] Not tested.

specific reactions. Two laboratories (D and E) conducted immunofluorescence tests on all the stem samples. Lab D recorded the results as IFU/ml and estimated that $>10^4$ IFU/ml constituted a positive sample whereas Lab E recorded the results as number of fluorescing cells per microscope field. Samples with an average of ≥ 5 cells per field were considered positive. On this basis, results from the two laboratories were in agreement for 84.4% of the samples (Table 5) and of these 96.6% agreed with the ELISA consensus reading. Individually, 90.7 and 87.9% of the immunofluorescence determinations by Labs D and E, respectively, were in agreement with the consensus ELISA determinations (Table 5).

Confirmation of serological tests by eggplant bioassay and by isolation of the pathogen was performed by Labs B and C, respectively. However, because the samples shipped for the comparative ELISA test were preserved with iodine, fresh stem tissue adjacent to that used for the ELISA sample had to be used as a source of viable bacterial cells. Differences in bacterial population density between the ELISA sample and the tissue available to Lab C for isolation were evident. Only 42% of the stems positive by consensus ELISA and by immunofluorescence in Labs D and/or E were positive in the immunofluorescence test done by Lab C. However, Lab C successfully isolated *C.m. sepedonicus* from 63% of the stems it found positive by immunofluorescence (Table 6). The bacterium was not isolated from any stems that were immunofluorescence negative. Although the presence of the ring rot

Table 7. Eggplant bioassay on stems of three potato cultivars grown from seed inoculated with three levels of *C.m. sepedonicus* and sampled on four dates; number of positive eggplants out of twelve[a] (immunofluorescence results in brackets)[b].

Treatment	Days after planting			
	69	83	99	111
Red Pontiac				
Check	0 (−,−)	0 (−,−)	0 (−,−)	0 (−,−)
Log 2	0 (+,−)	0 (−,+)	0 (−,−)	0 (+,−)
Log 4	0 (−,−)	0 (−,−)	4 (+,+)	12 (+,+)
Log 6	3 (+,+)	7 (+,+)	8 (+,+)	0 (+,+)
Russet Burbank				
Check	3 (−,−)	1 (−,−)	3 (−,−)	0 (−,−)
Log 2	NA[c]	NA	NA	NA
Log 4	0 (−,+)	0 (−,−)	0 (−,−)	0 (+,−)
Log 6	0 (−,+)	1 (−,−)	6 (−,+)	0 (+,−)
Désirée				
Check	0 (−,−)	0 (−,−)	0 (−,−)	0 (−,−)
Log 2	NA	NA	0 (−,−)	0 (−,−)
Log 4	0 (−,−)	0 (−,−)	3 (−,+)	0 (−,−)
Log 6	1 (−,+)	6[d] (+)	11 (+,+)	6 (+,+)

[a]Two potato stems were each tested in six eggplants.
[b]Immunofluorescence results for the two potato stems (rated + when mean number of cells per microscope field × dilution was ≥10, and − when counts were <10).
[c]Samples not available.
[d]Only one stem sample was tested on six eggplants.

pathogen could be confirmed by isolation in only some of the serologically positive stems, this was not surprising considering the slow growth characteristic of *C.m. sepedonicus* and the unavailability of good selective media. Furthermore, viability of bacterial cells may have decreased during shipment.

The eggplant bioassay has been used for sensitive detection of *C.m. sepedonicus* and facilitates isolation of the bacterium. The sensitivity of 100 cells/ml determined by Olsson (1976) and corroborated by Lelliott & Sellar (1976) for pure cultures was not attained in tests with infected plant material (Zeller & Xie, 1985; Janse & Van Vaerenbergh, 1987). Sixty-six percent of the stems tested in eggplant and found positive by consensus ELISA were positive in the eggplant assay (Table 7). Of the remaining 34% that were negative in the bioassay, more than one half (59%) were also negative in the immunofluorescence test done by Lab B.

Eight stems found to be positive by immunofluorescence by Lab B failed to give a positive eggplant test. The apparent low sensitivity of this test may have resulted from the growing conditions of the plants, which can influence sensitivity to infection by *C.m. sepedonicus* (Janse & Van Vaerenbergh, 1987). Surprisingly, seven eggplants inoculated with stem samples from check plants developed symptoms typical of *C.m. sepedonicus* infections (Table 7), perhaps from cross-contamination.

Alternatively other microorganisms in the sample could have caused typical wilt symptoms (Olsson, 1976; Persson & Janse, 1988).

Our results confirm that the ELISA test can give reproducible results for the detection of *C.m. sepedonicus* in plant samples. Although variation in ELISA data between laboratories was slightly higher than within laboratories, agreement in the results (Table 3) was as good as agreement in immunofluorescence data (Table 5). However, immunofluorescing cells were present in more samples than were considered positive in ELISA in these tests. Using the same monoclonal antibodies as we used, Gudmestad et al. (1991) found ELISA to be as sensitive as immunofluorescence when the accuracy of the serological tests was confirmed by isolation of the bacterium. The isolation and bioassay tests that could be carried out within the scope of this project were in general agreement with the serological test results.

As the ELISA procedure is particularly suitable for screening large numbers of samples, acceptance and implementation of this technique as an indexing tool for bacterial ring rot is likely to enhance the practicality of screening seed potato lots. By using two serological tests with monoclonal antibodies directed to two different antigens, the chances of false positive determinations would be reduced. Discrepancies between the two serological tests generally occurred with samples that had ELISA or immunofluorescence readings near the positive/negative threshold; these cannot be graded unequivocally as disease-free or contaminated with *C.m. sepedonicus*. By adjusting the threshold, it should be possible optimize sensitivity or specificity as required by operational standards.

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References

Anonymous, 1987. Scheme for the detection and diagnosis of the ring rot bacterium *Corynebacterium sepedonicum* in batches of potato tubers. EUR 11288. Office for Official Publications of the European Communities, Luxembourg, 21 pp.

Corbière, R., L. Hingand & B. Jouan, 1987. Application des méthodes ELISA et immunofluorescence pour la détection de *Corynebacterium sepedonicum*: réponses variétales de la pomme de terre au flétrissement bactérien. *Potato Research* 30: 539 – 549.

De Boer, S.H. & J.W. Hall, 1988. An automated microscope system for estimating the population of *Corynebacterium sepedonicum* cells labelled with monoclonal antibodies in immunofluorescence. *Canadian Journal of Microbiology* 10: 215 – 220.

De Boer, S.H. & M. McCann, 1990. Detection of *Corynebacterium sepedonicum* in potato cultivars with different propensities to express ring rot symptoms. *American Potato Journal* 67: 685 – 694.

De Boer, S.H., A. Wieczorek & A. Kummer, 1988. An ELISA test for bacterial ring rot of potato with a new monoclonal antibody. *Plant Disease* 72: 874 – 878.

De Boer, S.H., J.D Janse, D. Stead, J. Van Vaerenbergh & A.R. McKenzie, 1992. Detection

of *Clavibacter michiganensis* subsp. *sepedonicus* in potato stems and tubers grown from seed peices with various levels of inoculum. *Potato Research* 35: 207 – 216.

Gudmestad, N.C., D. Baer & C.J. Kurowski, 1991. Validating immunoassay test performance in the detection of *Corynebacterium sepedonicum* during the growing season. *Phytopathology* 81: 475 – 480.

Janse, J.D. & J. Van Vaerenbergh, 1987. Interpretation of the EC method for the detection of latent *Corynebacterium sepedonicum* infections in potato. *European Plant Protection Organization Bulletin* 17: 1 – 10.

Lelliott, R.A. & P.W. Sellar, 1976. The detection of latent ring rot (*Corynebacterium sepedonicum* (Spieck. et Kotth.) Skapt. et Burkh.) in potato stocks. *European Plant Protection Organization Bulletin* 6: 101 – 106.

Olsson, K., 1976. Experience of ring rot caused by *Corynebacterium sepedonicum* (Spieck. et Kotth.) Skapt. et Burkh. in Sweden. Particularly detection of the disease in its latent form. *European Plant Protection Organization Bulletin* 6: 209 – 219.

Persson, P. & J.D. Janse, 1988. Ring rot-like symptoms in *Solanum melongena* caused by *Erwinia chrysanthemi* (potato strain) after artificial inoculation. *European Plant Protection Organization Bulletin* 18: 575 – 578.

Saettler, A.W., N.W. Schaad & D.A. Roth, 1989. Detection of bacteria in seed and other planting material. American Phytopathological Society Press. St. Paul, Minnesota, 122 pp.

Samson, R. & F. Poutier, 1979. Comparaison de trois methodes d'identification de *Corynebacterium sepedonicum* dans les tuberules de pomme de terre. *Potato Research* 22: 133 – 147.

Zeller, W. & Y. Xie, 1985. Studies on the diagnosis of bacterial ring rot of potatoes. I. Pathogenicity test on eggplants. *Phytopathologische Zeitschrift* 112: 198 – 206.

Zielke, R. & I. Kalinina, 1988. Ein Beitrag zum Nachweis von *Clavibacter michiganensis* subsp. *sepedonicus* (Spieckermann & Kotthoff) Davis et al. im Pflanzengewebe mit dem Mikroliter-ELISA-Verfahren. *Zentralblatt für Mikrobiologie* 143: 5 – 16.