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Characteristics of immunofluorescence microscopy and of dilution-plating to detect Pseudomonas syringae pv. phaseolicola in bean seed lots and for risk assessment of field incidence of halo blight*

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Abstract

Routine laboratory testing of 710 bean seed lots from various origins for *Pseudomonas syringae* pv. phaseolicola (Psp) with immunofluorescence microscopy (IF) showed that 27.5% of the seed lots (five subsamples of 1000 seeds tested per sample) contained two or more IF-positive cells in a total of 500 microscope fields (magnification 500×). Simultaneously performed dilution-platings of IF-positive subsamples on King's medium B confirmed presence of Psp for one-third of these IF-positive seed lots. The 'grey area' of disagreement between both laboratory tests was studied by comparison of test data and by field trials.

The number of IF-positive cells per subsample was positively correlated with isolation and identification of Psp ($R = 0.85$). The detection level of IF was ca. 10^2 Psp cells per ml of undiluted subsample extract. The detection level of Psp by isolation on King's medium B was variable, being inversely related with the saprophyte to Psp ratio. The high sensitivity of IF was in part due to high percentages of dormant or dead IF-positive cells in the sample extract. Field trials over two years with 10 000 seeds per seed lot, showed disease incidence for 9 of the 22 seed lots. Of ten IF-positive lots with five positive subsamples per sample, nine were positive in the field test plot (the negative lot gave primary infection spots of Psp when used for commercial growing). By isolation, seven of these ten IF-positive lots were positive. Of the five IF-positive lots with two or less positive subsamples, isolation and field trial were both negative. Based on data on seed transmission from literature, field incidence was unlikely for these five samples in a 10 000 seeds field trial. All seven IF-negative lots were negative in the field trial. The value of IF and isolation for indexing bean seed lots for Psp is discussed.

Additional keywords: Phaseolus vulgaris, *Phaseolus coccineus*, bush bean, runner bean, method evaluation, grey test area, detection level, saprophytic interference, predictive value, diagnostic sensitivity, diagnostic specificity, analytical sensitivity, analytical specificity.

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Introduction

Methods for the detection of seed-borne bacteria are reviewed by Schaad (1982, 1989). Both dilution-plating and immunofluorescence microscopy (IF) are widely used for this purpose. Both methods may have disadvantages. In dilution-plating false-negatives may occur due to poor or no growth of the target organism or from its suppression by saprophytes in the seed sample. The major risk of a serological method like IF is a false-positive result, due to a non-specific serological reaction with a non-target organism present in the sample. False-negative results may be obtained if the antiserum is not sufficiently tested against a panel of strains representing the various serotypes of the pathogen. Dead cells of the target organism will give a positive reaction in IF. The different risks on a false-positive or a false-negative test result for isolation and for IF will result in a so called 'grey area' of disagreement between both laboratory methods for a series of samples.

The need for high diagnostic sensitivity and specificity together with the ability to index large numbers of samples in a short time at low costs is discussed by Sheppard et al. (1986). For seed-borne diseases such as halo blight with a certain level of tolerance in various countries (Trigalet and Bidaud, 1978; Taylor et al., 1979b), it will be necessary to quantify the results of the laboratory assay and to investigate the relationship between the results of the actual laboratory assay used and the actual transmission of the pathogen from the seed to the seedlings under field conditions.

In our studies we have investigated large numbers of bean seed samples for *Pseudomonas syringae* pv. *phaseolicola* (Psp) with a direct IF assay. The seed lots with IF-positive results were further tested by dilution- plating on a general plating medium and identification procedures to evaluate the significance of the IF-test and to analyze in detail some of the critical factors involved in both laboratory assays in regard to this grey zone of disagreement. Field trials were performed in two separate years with a selection of samples to study the predictive value of both laboratory assays in relation to field incidence of the disease.

Materials and methods

Seed samples. The 710 samples involved in this evaluation were submitted under code for routine indexing for Psp at the Government Seed Testing Station (RPvZ) in the period 1981 to 1984. They originated from imported seed lots from various countries and from samples supplied by seed companies. The test sample, and the five subsamples of 1000 seeds each per test sample, were prepared according to ISTA-rules for germination tests (ISTA, 1985). Each subsample was further analyzed independently.

Laboratory assay. Subsamples of 1000 seeds were incubated in tap water and analyzed after 6 h at 6 °C according to the standardized procedure (Van Vuurde and Van den Bovenkamp, 1987; 1989). IF-smears were made by spreading 50 µl of each subsample over an area of 6 mm diameter of a microscope slide. Samples with more than one IF-positive cell in a subsample (100 microscope fields) or totally two or more IF-positive cells in the five subsamples (500 microscope fields) were analyzed again after a total incubation of 24 h at 6 °C with IF and with dilution-plating. Readings of the IF-preparations were done at 500 times magnification (objective 40×, ocular

10× with field coefficient 18, tube magnification factor 1.25), resulting for 100 microscope fields in a screening of ca. 10 mm^2 and 17.7 μl of the seed extract. For the subsamples positive in IF after 6 h, dilution-series on King's medium B were prepared simultaneously with the preparation of the IF-preparations after 24 h incubation. Aliquots of 500, 150, 50 and 5 μl were plated for each subsample and incubated for 4 days at 28 °C. Suspected colonies, selected on the basis of production of a blue or blue-green fluorescent pigment, were purified and identified by the oxidase test and pathogenicity testing on bean cotyledons (Van Vuurde and Van den Bovenkamp, 1987). The number of colony forming units (cfu) of Psp colonies and the total number of cfu were calculated according to ISO 7218 (1985).

Field experiments. Seed lots, representing various cultivars and origins, were used for the field trials in 1984 (13 seed lots) and 1987 (9 seed lots). The selection was based on the results of the standard routine laboratory assay, performed under code at the Government Seed Testing Station. These series of seed lots with different rates of Psp contamination in IF and/or dilution-plating were sown in experimental field plots in Haelen (sandy soil, S.E. part of the Netherlands). For each sample, 10 000 seeds were sown in rows with an average distance of 30 cm between the rows, and of 10 cm between the plants. The fields were inspected twice a week up to two-and-a-half weeks after emergence. The number of primary infection spots in a field was determined by the number of individual seedlings with symptoms of a seedborne nature and the number of spots with secondary infected plants around a rotten or dead seedling. Seedlings with doubtful symptoms at two-and-a-half weeks after emergence were analyzed at the RPvZ for the presence of Psp by isolation followed by a pathogenicity test and IF with pure culture suspensions.

Results

Laboratory indexing. The results of the testing of 710 seed lots with IF and dilution-plating are summarized in Table 1. About 55% of all the samples were completely negative in IF after 6 h of incubation for all five subsamples of 1000 seeds (0 IF-positive cells in 500 microscope fields). For 17% of the samples we detected one IF-positive cell in 100 microscope fields in only one of the five subsamples. These 72.5%

Table 1. Results of routine indexing of imported and trade seed lots with IF and with dilution-plating on King's medium B in the period 1981 - 1984.

Samples	Number	Percentage of total
IF negative	392	55.2
IF 1 cell in 5 × 100 microscope fields	123	17.3
IF 2 or more cells in 5 × 100 microscope fields		
– negative with dilution plating	125	17.6
– positive with dilution-plating	70	9.9
Total number of samples	710	100.0

of the samples were reported negative after the IF-test. The 195 IF-positive samples with two or more positive cells in 500 microscope fields were further investigated with dilution-plating. For these 195 IF-positive samples, the presence of Psp could only be confirmed by dilution-plating for 70 samples (9.9% of all test samples). The 'grey area' of disagreement amounted to 17.6% of all the tested samples, for which the presence of two or more IF-positive cells could not be confirmed by actual isolation of Psp.

The correlation between the number of IF-positive cells in a subsample and the possibility to isolate Psp was analyzed for subsamples from samples with two or more IF-positive cells in 500 microscope fields. This included subsamples with one IF-positive cell from samples with two or more IF-positive subsamples. The subsamples were classified in six classes according to the number of IF-positive cells in 100 microscope fields (Table 2). Most subsamples were in the classes: 1 to 5, 100 to 500, and 500 to 2500 IF-positive cells per 100 microscope fields. Results of Table 2 demonstrate that 26.9% of the positive subsamples contained less than five positive cells per 100 fields. For only 8.8% of the subsamples of this class, the IF-positive results were confirmed by isolation of Psp from that subsample. Psp could be isolated from 76.2% of the subsamples with more than 2500 cells per 100 fields. Increase in the numbers of IF-positive cells per subsample, expressed as the mean of the various classes of Table 2, correlated with an increase of the percentage of subsamples from which Psp could be isolated ($R = 0.85$).

For an analysis of the 'grey area' between results of IF and of dilution-plating we have classified IF-positive subsamples according to the same six classes used in Table 2, together with the estimated total number of saprophyte and Psp colony-forming units per ml based on ISO calculations. This was done for the samples of 1983 and 1984, as only for these years data were available on the number of saprophytes of all plates of the dilution series of each subsample. Data are presented for the classes with

Table 2. Comparison between IF-positive subsamples classified according to the number of IF-positive cells in 100 microscope fields in a subsample extract of 1000 seeds and the possibility to isolate *Pseudomonas syringae* pv. *phaseolicola* on King's medium B from that subsample.

	Number of IF-positive cells in 100 fields					
	1-4[1]	5-20	21-100	101-500	501-2500	>2500
Total number of IF-positive subsamples	57	30	10	48	46	21
Class frequency(%)	26.9	14.2	4.7	22.6	21.7	9.9
Subsamples negative in dilution-plating	52	25	7	31	16	5
Subsamples positive in dilution-plating	5	5	3	17	30	16
Positive in IF and dilution-plating (%)	8.8	16.7	30.0	35.4	65.2	76.2

[1] A subsample with one IF-positive cell per 100 fields originates from a sample with at least two subsamples with one or more positive cells per 100 fields.

the lowest and the highest correlation between IF and dilution-plating; viz. 1 - 5 and more than 2500 IF-positive cells per 100 fields in Table 3A and 3B, respectively.

From the class with 1 - 5 IF-positive cells in Table 3A, Psp could only be isolated from 2 out of 19 subsamples. In subsample A5 only 2 IF- positive cells were found in 100 fields, but isolation of Psp was possible due to the low incidence of saprophytic bacteria allowing a reliable reading of the dilution-plates with 150 and 500 µl undiluted seed extract. Subsample A5 contained 10 Psp cfu per ml and ca. 17 times more saprophytes. Subsample A13 with 4 IF-positive cells in 100 fields contained 400 Psp cfu per ml and 19 times more saprophytes. Attempts to isolate Psp were negative for several subsamples with comparable numbers of saprophytes.

Results in Table 3B show that for subsamples with more than 2500 cells per 100 microscope fields. Compared with Table 3A, Psp was isolated from a much higher

Table 3. Comparison of the numbers of IF-positive cells in 100 microscope fields to the number of cfu of *P. syringae* pv. *phaseolicola* (Psp) and saprophytic microorganisms on King's medium B for two frequency classes of IF-positive cells.

A. Subsamples with 1-4 cells per 100 microscope fields

Sub-sample no.	IF (cells per 100 microscope fields		Dilution-plating (cfu/ml)	
	6 h	24 h	Psp	saprophytes
A1	1	0	–[1]	60000
A2	1	0	–	4000
A3	1	0	–	800
A4	2	0	–	9090
A5	2	0	10	170
A6	2	0	–	200
A7	3	0	–	72700
A8	3	0	–	50000
A9	3	0	–	420
A10	3	0	–	440
A11	3	0	–	2750
A12	3	0	–	400
A13	4	0	400	7600
A14	4	0	–	380
A15	4	0	–	550
A16	4	0	–	730
A17	4	0	–	820
A18	4	0	–	560
A19	4	0	–	400

B. Subsamples with more than 25 cells per microscope field

Sub-sample no.	IF (cells per microscope field)		Dilution-plating (cfu/ml)	
	6 h	24 h	Psp	saprophytes
B1	30	3	600	350
B2	30	1	—	10450
B3	30	6	2980	5200
B4	30	2	110	12620
B5	30	3	–	630
B6	40	1	–	190
B7	40	25	>200[2]	>60000
B8	50	1	600	2200
B9	50	10	30000	–
B10	60	2	20000	–
B11	60	10	13000	1000
B12	>60	2	60000	–
B13	>60	5	–	180
B14	>60	2	1400	780
B15	>60	10	1600	12400

[1] – = Psp or saprophytes not found .

[2]All dilution-plates were covered with a smear of colonies. Presence of at least one colony on the 5 µl plate could be confirmed.

percentage of these subsamples. The Psp colonies were sometimes present in higher numbers than the saprophyte colonies on the dilution plates (B11, B14), or present as the only colony type (B9, B10, B12). Psp concentrations were in the range of 10^3 to 10^5 cfu's per ml for the subsamples B3, B9, B10, B11, B12 with relatively low saprophyte numbers. Subsamples B2 and B5 with relatively high numbers of saprophytes and subsampless B6 and B13 with relatively low numbers of saprophytes were negative for Psp in dilution-plating. Attempts to isolate a cross-reacting bacterium from these plates failed. The IF results for 6 h compared with 24 h sample incubation showed a decrease in the number of IF-positive cells with a factor less than 2 (B7) to 50 times (B8). The correlation of the log number of IF-positive cells after 6 h and 24 h with the log number of Psp cfu per ml estimated for positive subsamples with a low interference of saprophytes (A5, B1, B9, B10, B11, B12) was 0.934 and 0.859, respectively.

Generally, most subsamples were found positive for the 50 and 5 μl platings. For a series of 23 IF- and isolation-positive subsamples (data not shown), Psp was isolated from 500, 150, 50 and 5 μl plates for 8, 8, 15 and 18 subsamples, respectively. The 150 μl plates were positive for 2 subsamples which were negative for the higher dilutions. The 500 μl platings did not add positive subsamples, which were negative in the higher dilutions.

The estimated number of Psp cfu per ml found on the dilution series of the positive subsamples listed in Table 3A and 3B varied from 10 (A5) to 60 000 (B12) per ml. The estimated number of saprophyte colonies varied between 0 and 60 000 (A1). The highest saprophyte to pathogen ratio for a subsample from which Psp could be isolated was 115 (B4).

Comparison between laboratory assays and field incidence. In the 1984 field trial, eleven of the thirteen samples were grown at the experimental field under rather favourable climatological conditions for the pathogen (wind and rain) during the first week after seedling emergence. Two seed lots (84-6 and 84-12) were sown two weeks later under less favourable (dryer) weather conditions for disease development. Weather conditions in 1987 were less favourable than in 1984 for the development of halo blight symptoms during the first month after emergence.

The results of the laboratory test and the field incidence of Psp are summarized in Table 4. The ten seed lots with all five subsamples positive in IF (seed lots 84-1 to 8 and 87-1 and 87-2 in Table 4) show a good correspondence with isolation of Psp and with primary spots of halo blight in the field trial, resp. 70% and 90%. Seed lots with five IF-positive subsamples with 20 or more cells per 100 microscope fields (84-1 to 84-5, 87-1) gave between 5 and 30 primary infection spots. Seed lot 84-7 was negative for isolation of Psp and in the field trial, but primary halo blight spots were recorded when this seed lot was used for commercial bean growing in 1984. Seed lots 84-5 and 87-2 showed primary infection of halo blight in the field, but were negative with isolation. For these two samples high numbers of saprophytes were recorded for the dilution series.

Seed lots 84-9, 84-10, 87-3 and 87-4 with two out of five, and seed lot 87-5 with one out of five IF-positive subsamples, were negative in isolation and the field trial. All seven seed lots which were negative in IF for the five subsamples were also negative in the field trial.

Table 4. Comparison between laboratory seed assays and incidence for *P. syringae* pv. *phaseolicola* in field trials in 1984 and 1987.

No. sample	IF, 5 subsamples of 1000 seeds[1]					Dilution-plating	Primary infected spots in field trial 10 000 seeds
	1	2	3	4	5		
A. Field trial 1984							
84-1	++	++	++	++	++	+	24
84-2	+	+	++	++	++	+	8
84-3	+	+	+	++	++	+	12
84-4	+	+	+	+	++	+	30
84-5	+	+	+	+	+	−[2]	5
84-6	±	+	+	+	+	+	1
84-7	±	±	+	+	+	−[2]	0/+[3]
84-8	±	±	±	±	+	+	1
84-9	−	−	−	±	+	−[2]	0
84-10	−	−	−	±	±	−	0
84-11	−	−	−	−	−	N.D.	0
84-12	−	−	−	−	−	N.D.	0
84-13	−	−	−	−	−	N.D.	0
B. Field trial 1987							
87-1	+	++	++	++	++	+[2]	9
87-2	±	±	±	+	+	−[2]	2
87-3	−	−	−	±	++	−[2]	0
87-4	−	−	−	±	+	−[2]	0
87-5	−	−	−	−	+	−[2]	0
87-6	−	−	−	−	−	N.D.	0
87-7	−	−	−	−	−	N.D.	0
87-8	−	−	−	−	−	N.D.	0
87-9	−	−	−	−	−	N.D.	0

[1] Code for numbers of IF-positive cells per subsample in 100 microscope fields: − = 0; ± = < 20; + = 20 to 500; ++ = > 500.
[2] High numbers of saprophytes on the dilution plates.
[3] Primary infection spots in production field.

Discussion

Parameters for evaluation of a test system. Sheppard et al. (1986) discussed several test parameters which should be considered in the development of a test system. The diagnostic sensitivity of a test is described as the ability of a test to correctly identify a seed lot with a specified pathogen when the disease status of the seed lot is known. The diagnostic specificity is defined as the ability to correctly identify healthy seed lots, i.e. those free from the specified pathogen when the disease status is known. The analytical sensitivity refers to the theoretical lowest concentration of the target per unit sample which can be detected without interference of biotic or abiotic factors of the sample. The term analytical specificity is e.g. used to define the degree of immunolo-

gical cross-reactivity in IF. The predictive value represents the probability that a positive or negative result is truly reflective of the disease status in a given population. We have used these parameters as a tool in the discussion on the advantages and disadvantages of IF and of isolation to detect target bacteria and to discuss a strategy for seed testing in practice.

Characteristics of IF. The analytical sensitivity of IF for a subsample was one cell in 100 microscope fields in our study. This corresponds to a quantity of 17.7 μl sample extract and an analytical sensitivity of 55 cells per ml as calculated according to Van Vaerenbergh (1984). With an assumed background of one cell in 100 microscope fields, as used for the seed lots with one subsample out of five positive and one cell per 100 fields, the detection level for an IF-positive subsample was ca. 10^2 target cells per ml. This background may originate from a Psp cell or a cross-reacting bacterial cell from the sample or the cross-contamination with Psp from other sources during the processing of seed lots, seed test samples, and IF-preparations. Test results obtained from over 700 seed lots showed that the 55% did not contain a single IF-positive cell in the total of 500 microscope fields checked per sample. This indicates that the general level of non-target cells which give a positive reaction in IF is very low in our test system for Psp. A similarly low background was also found for detecting *Xanthomonas campestris* pv. *phaseoli* in bean seeds (Van Vuurde et al., 1983). In comparison, Miller (1984) found a rather consistent background of 2.5×10^3 to 5×10^3 non-specific IF-positive cells in testing heel-end extracts of 40 samples of healthy seed potatoes for *Clavibacter michiganensis* subsp. *sepedonicus*.

Of the seed lots in Table 1, 123 contained only one positive cell in the 500 fields. Arbitrarily, this was taken as the background level, mainly for practical reasons, i.e. the amount of labor involved in additional confirmation tests in regard to the limited chance on isolation Psp or a cross-reacting non-target bacterium from these seed lots. Therefore these samples were not further investigated and were reported negative. The 195 samples which contained two or more IF-positive cells in 500 fields (including some samples with only two subsamples with one IF-positive cell in 100 fields) were further analyzed for the significance of low numbers of IF-positive cells in regard to the presence of Psp. For these subsamples the number of IF-positive cells ranged from one to over 6000 in 100 fields (Table 3B).

Six hours extraction was found to be optimal for the release of high numbers of Psp cells from bean seeds and for handling of the test samples for IF in routine indexing procedures (Van Vuurde et al., 1983). The strong decrease in the number of IF-positive cells between the average optimal extraction time of 6 h with that of 24 h incubation (Table 3B) indicates the presence of high numbers of dead Psp cells which broke down during the 24 h soaking at 6 °C.

Characteristics of isolation. Isolation on Kings's medium B and identification of Psp-type colonies was worked out in detail by Taylor (1970) and was successfully used in the UK for small scale indexing of bean seed. Low numbers of saprophytes were in general present in the samples of runner beans used by Taylor, as these beans often ripen under dry conditions unfavourable for saprophyte development (J.D. Taylor, personal communication). The bean seed lots tested in our indexing program were bush bean cultivars with pods ripening in the canopy close to the soil surface. When

ripened and harvested under humid conditions, and especially when this coincided with damage of pods or seeds by equipment, hail, or pathogens, high numbers of saprophytes could be found on the seeds. The varying number and competitive properties of the saprophytes in the dilution-series has clearly affected the sensitivity of isolation in our experiments (Table 3A,B) and may have caused false-negative test results. One rotten seed may contain over ten million colony-forming bacteria (Taylor et al., 1979a), which in the case of competitive saprophytes on King's medium B, can prevent the isolation of Psp from a test sample of 1000 seeds with several Psp infected seeds. The number of Psp cfu found per ml subsample extract varied from 10 to over 60 000 (Table 3). The analytical sensitivity was 2 cfu per ml subsample extract (500 μl per plate). However, because of saprophytic interference reliable reading according to ISO standards was for most subsample extracts only possible for the 50 or 5 μl platings.

Comparison of IF and dilution-plating. Of the 710 samples tested, 17.6% was classified in the grey zone of discrepancy between results obtained by IF and isolation (Table 1). Of the IF-positive samples with two or more IF-positive cells in a total of 500 microscope fields, only 36% was also positive with isolation. A clear positive correlation was found between increasing numbers of IF-positive cells per subsample (100 microscope fields) and the possibility to isolate Psp from that subsample (Table 2). Data from Table 3A,B show that IF may be ca. 100 times more sensitive than dilution-plating for several samples. The 'grey area' of discrepancy between the results of both laboratory tests was explained from the data in Table 3. Important factors are the variable detection level of isolation due to the interference of saprophyte colonies which may have overgrown or inhibited Psp in the dilution series (Table 3A,B), and the general high incidence of dead or non-germinating Psp cells which still give a positive IF reaction (e.g. Table 3B). Furthermore, cross-reacting microorganisms may have been a factor contributing to the 'grey area'. Attempts to isolate a cross-reacting microorganism from plates of subsamples which were negative for Psp isolation but with several IF- positive cells per microscope field were negative for the non-selective King's medium B. Based on the zero background, viz. 0 cells in 500 fields, in 55% of the samples, the specificity check of the antiserum (Van Vuurde et al., 1983), and the failure to isolate a cross-reacting microorganism, we expect that the incidence and the number of IF-positive cells of non- target organisms in an average bean seed sample is low or zero. However, a false positive IF-result due to a cross-reaction with a non-target organism with similar cell wall antigens can not be excluded completely for the IF-positive samples in which the presence of Psp could not be confirmed by isolation.

Taylor et al. (1979a) found that the number of viable Psp cells in contaminated bean seed decreases with a factor 250 per annum. Other factors than storage conditions such as chemical treatment of seed lots (Taylor and Dudley, 1977), and/or the presence of chemicals from the test sample in the seed extract, may also have reduced the number of Psp cfu below the detection level. One of these factors or a combination of factors might be responsible for the negative result of isolation of Psp from some samples with high numbers of IF-positive cells and low numbers of saprophytes (e.g. samples B6 and B13 in Table 3B). This could not be further investigated as no data on these factors were available for the submitted samples.

Relationship between laboratory results and field incidence. The percentage of

infected seeds in a sample of 5000 seeds, which is subdivided in 5 × 1000 seeds can be estimated with the most probable number (MPN) method (Taylor, 1970; Taylor and Phelps, 1984). For two out of five IF-positive subsamples this is estimated at 0.05% (upper limit 0.16%). Based on ca. 10% seed transmission for Psp (Taylor et al., 1979b), primary spots of halo blight can only reliably (probability 95%) be expected in a field trial with 10 000 seedlings for seed lots with more than 0.3% infected seeds. Based on these data, only the seed lots with five IF-positive subsamples were expected to give primary spots of halo blight in the field. On the basis of the results in the field trial with 10 000 seeds, the estimated percentage of seed transmission of 10%, calculations of the diagnostic sensitivity, diagnostic specificity and the predictive values of a positive and negative result of IF and isolation can be made. The diagnostic sensitivity was 100% and 78% for IF and dilution-plating, respectively. The diagnostic specificity was 89% and 100% for IF and dilution-plating, respectively. The predictive value of a negative result was 100% and 87% for IF and dilution-plating (including the IF-negative samples which were not plated), respectively. The predictive value of a positive result was 90% and 100% for IF and dilution-plating, respectively.

Strategies for testing bean seed lots. The high and consistent analytical and diagnostic sensitivity of IF, due to the detection of viable and dead cells and the lack of interference of saprophytes, and its suitability for routine application, make IF attractive for screening seed lots for Psp. However, the diagnostic specificity may vary because of detection of dead cells and the low rate of seed-transmission. The analytical specificity is considered to be high since no cross-reacting bacteria could be isolated. The favourable characterisitics of isolation are its high analytical and diagnostic specificity, because viable Psp-cells are detected and test results will be 100% reliable after proper identification including a check for pathogenicity.

Our test system is based on the use of IF as a screening and of isolation to confirm IF-positive seed lots. A special complication in the combined use of both methods is the interpretation of contradictory test results, in our test series IF-positive and isolation-negative test samples. The value of both IF and isolation was demonstrated by the high correlation of each test with disease incidence in the field experiment. The advantages of the high diagnostic sensitivity of IF to screen for 'positive' samples and the high diagnostic specificity of isolation as a tool to distinguish between true and false positive samples made reliable indexing of 82% of the samples possible (Table 1). The interpretation of the test results in the grey test area (18% of the 710 samples) is also dependent on whether Psp is considered a quality disease or a quarantine disease. For disease free production of beans e.g. in Psp-free areas, the IF-test with a high diagnostic sensitivity will be better able to indicate the potential risk of a seed lot on disease incidence (viz. Table 4, samples 84-5, 84-7, 87- 2). The grey area can be strongly reduced by accepting a higher number of IF-positive cells per subsample in combination with the number of IF- positive subsamples before a seed lot is declared positive. However, such a 'cut-off' level will affect the prediction of a disease risk in a commercial production field with more than 10 000 plants (Table 4, sample 84-12). When a prediction for 'no disease' is required, IF is the most suited test. On the other hand, isolation detects true Psp-positive seed lots with a large risk on disease incidence in the field.

The further development of test systems for Psp in bean seeds should aim at a narro-

wing of the 'grey area'. Prospects to improve the detection of Psp are: using monoclonal or polyclonal antibodies prepared against a typical and pathogenicity-related antigen of Psp and the developement of (semi-)selective media for Psp. The (semi-) selective MSP medium (Mohan and Schaad, 1987) was compared with King's medium B for detection of Psp and showed to be promising (A.A.J.M. Franken and M. Hooftman, unpublished results). When testing a series of 29 IF-positive subsamples with both media, 23 subsamples were found positive with both King's medium B and MSP. Two subsamples were found positive with King's medium B, but not with MSP. Four subsamples were found positive with MSP, but not with King's medium B. Alternatively, the development of new detection systems based on DNA-hybridization (Schaad et al., 1989), possibly in combination with the polymerase chain reaction (PCR) or a direct combination of isolation and serology may have good potential for testing plant propagative material (Franken and Van Vuurde, 1990; Van Vuurde and Van Henten, 1983; Van Vuurde, 1987). Immunofluorescence colony staining (Van Vuurde and Roozen, 1990) with plant extracts mixed through the agar combines the advantages of the (analytical) sensitivity of IF and (analytical) specificity of isolation, and when applied at the level of microcolonies, even allows the distinction between dividing and dormant or dead cells (Van Vuurde, 1990).

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