Microdensitometer Measurements of Sequential Aerial Photographs of Field Beans Infected with Bacterial Blight

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Interested readers are referred to a companion article (on page 942 in this issue) titled: "Model for yield loss determination of bacterial blight of field beans utilizing aerial infrared photography combined with field plot studies."

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ABSTRACT

Optical density levels were determined from aerial photographs of healthy and bacterial (*Xanthomonas phaseoli*) blight-infected bean plots throughout the growing seasons in 1971 and 1972. Three films, Kodak Aerochrome Infrared 2443, Kodak Ektachrome MS Aerographic 2448, and Kodak Infrared Aerographic 2424 were used in 1971. Only Kodak Aerochrome Infrared 2443 was used in 1972. Changes in diseased plots were not apparent in the films exposed during July despite the onset of infection following inoculation of the plots in late June. Density levels between healthy and diseased plots showed obvious differences from 2 August 1972 and 5 August 1971 and throughout the growing season.

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Differences between diseased and healthy areas in certain crops can be demonstrated by comparing optical density levels in aerial photographs made on some types of films. Manzer and Cooper (6) detected late blight of potato by infrared color aerial photography, and utilized optical density readings in an attempt to compare disease severity. In similar work, Jackson et al. (5) showed that optical densities were significantly ($P = 0.01$) correlated with direct field assessments of plant disease by plant pathologists. In both instances, evaluations were made from one photograph of the crop which was in the advanced stages of symptom expression.

The objective of this work was to ascertain if optical density measurements of sequential aerial photographs of plots of diseased and healthy field beans (*Phaseolus vulgaris* L.) made throughout the growing season could be utilized for remote assessment of disease severity or complement field observation methods. Assessments are now made by plant pathologists who examine diseased plants and estimate disease severity through the use of growth-stage and disease-assessment keys.

MATERIALS AND METHODS.—*Field plots.*—The field plots, design, assessment methods, and inoculation procedure for the production of the epiphytotic of bacterial blight caused by *Xanthomonas phaseoli* (E. F. Sm.) Dows. is reported in a companion paper (8).

Aerial photography.—Five flights were made over experimental plots in 1971 (20 and 27 July; 5, 12, and 19 August) and seven flights in 1972 (18 and 27 July; 2, 11, 21, and 29 August; 5 September) using a Bell-47G helicopter at an altitude of approximately 400 feet. Exposures were made with a boom-mounted Maurer 70-mm camera positioned for vertical photography. The camera was fitted with a 38-mm Biogon *f*/4.5 lens, which produced an approximate scale of 1:3,200 (i.e., size of image to distance on ground). Exposing rate was six frames per second, and the air speed was approximately 40 knots.

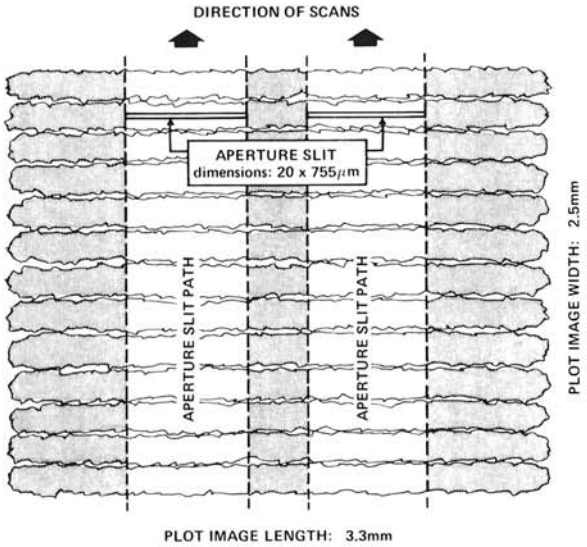

Fig. 1. Microdensitometry of aerial photographs of field bean plots infected with bacterial blight. Path of microdensitometer aperture across plot image showing slit positions and direction of scans.

TABLE 1. Flight dates, film types, and Wratten filters used in the photography of bacterial blight of field beans

	Kodak film type and Wratten filter no.		
Date	2448 None	2443 No. 12	2424 No. 89B
1971			
20 July		x	
27 July	x	x	x
5 August	x	x	x
12 August		x	
19 August	x	x	x
1972			
18 July		x	
27 July		x	
2 August		x	
11 August		x	
21 August		x	
29 August		x	
5 September		x	

Three film types were tested in 1971, Kodak Aerochrome Infrared film 2443, Kodak Ektachrome MS Aerographic film 2448 (both developed as positives in E-4 chemicals), and Kodak Infrared Aerographic film 2424 (developed as negatives in Kodak Versamat Type-A chemicals). Only Kodak Aerochrome 2443 was used in 1972. All films were exposed at a shutter speed of 1/500 second, with diaphragm openings and filters of *f*/5.6 for the 2443 film with a Wratten No. 12 filter; *f*/4.5 for the 2448 film without a filter; and *f*/8 with a Wratten No. 89B filter for the 2424 film.

The three films were not exposed simultaneously nor were all used on each flight (Table 1). However, the delay between flights on any one day was kept to a minimum by landing at the site to change film magazines, and immediately returning aloft. Photography was attempted only in the periods 0930-1100 hours and 1300-1430 hours, Eastern Standard Time, to eliminate the risk of hot spots and to obtain similar sun angles. The sun is never directly overhead at Ottawa (Lat. 45°23′, Long. 75°42′) and the solar altitude varies from 21°57′ to 36°32′ at 1200 hours Standard Time, from the first of June to the end of August.

Each photograph contained images of twelve plots, and exposures were made at different times throughout the growing season (Table 1). Due to poor drainage at the test site, only images of eight plots were analyzed from the 1971 series, and four from the 1972 series.

Density measurements.—The response of photographic materials to various wavelengths and intensities of light may be measured from the optical densities produced in them by suitable exposure and subsequent development. The optical density of monochrome films is determined by measuring the opacity of the blackened silver contained in the emulsion; with color films, the absorption characteristics of the dyes formed in the three light-sensitive layers determine the density to any one color produced by the combination of the dyes. Most commonly-used color photographic materials are composed of three layers sensitive to blue, green, and red wavelengths. In these layers during processing, dyes are formed which are complimentary to the exposing light; i.e. yellow, magenta, and cyan,

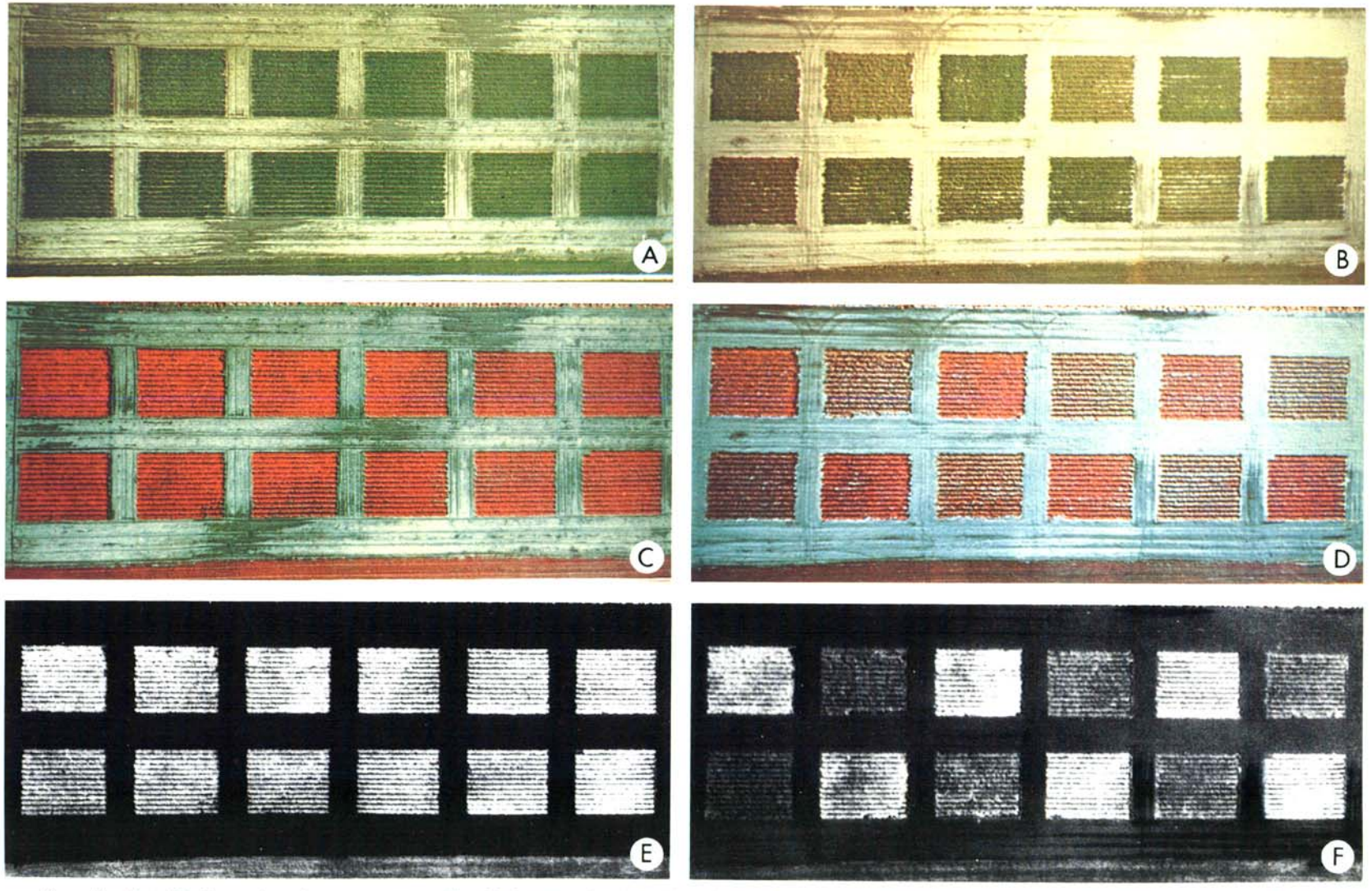

Figure 2 – (**A** to **F**). Comparisons between two sets of aerial photographs selected from five flight dates over the same 12-plot randomized block of field beans showing the progression of symptoms of bacterial blight infection. (**A, C** and **E**) exposed on 27 July 1971 and (**B, D** and **F**) exposed on 19 August 1971; using (**A – B**) Ektachrome MS 2448, (**C – D**) Aerochrome Infrared 2443 and (**E – F**) Infrared Aerographic 2424.

TABLE 2. Averaged[a] transmission densities from aerial photographs of control and bacterial blight-infected plots of field beans

	Kodak film type and densitometer filter no.					
	2448 (No. 93)		2443 (No. 29)		2424 (None)	
Date	Control	Infected	Control	Infected	Control	Infected
1971						
20 July	...	...	0.50	0.50	...	...
27 July	1.19	1.17	0.65	0.66	2.23	2.15
5 August	1.41	1.38	0.90	0.89	1.79	1.76
12 August	...	...	0.80	0.88	...	...
19 August	1.08	1.07	0.98	1.14	1.82	1.53
1972						
18 July			0.36	0.36		
27 July			0.38	0.38		
2 August			0.75	0.82		
11 August			0.67	0.72		
21 August			1.13	1.36		
29 August			0.46	0.66		
5 September			0.60	0.79		

[a]Averages of two scans each from four control plots and four infected plots for three types of film for each date in 1971, and two scans each from two control and two infected plots for one type of film for each date in 1972.

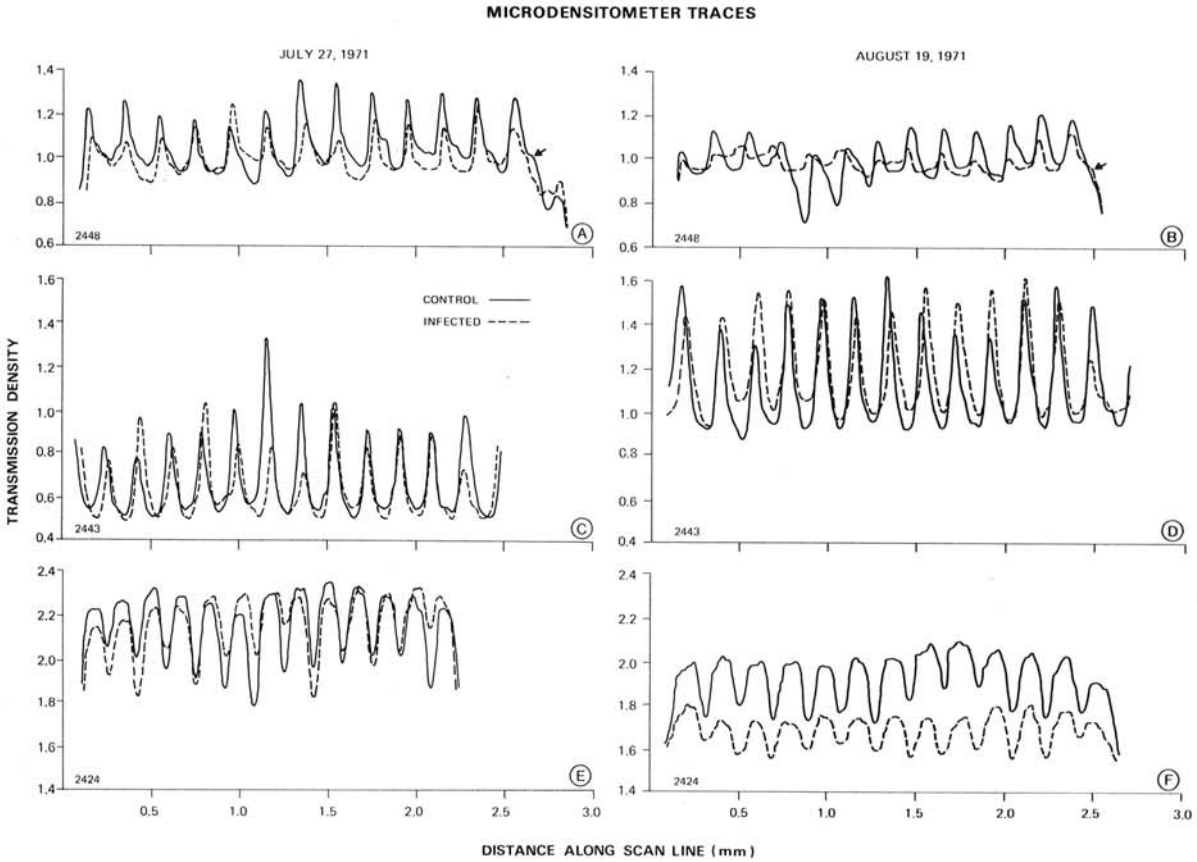

Fig. 3-(A to F). Microdensitometry of aerial photographs of field bean plots infected with bacterial blight. Microdensitometer traces selected for the same two dates as Figure 2 (**A, C and E**) 27 July 1971 and (**B, D and F**) 19 August 1971 showing transmission density comparisons between (**A and B**) Kodak Ektachrome MS Aerographic 2448 film, (**C and D**) Kodak Aerochrome Infrared 2443 film and (**E and F**) Kodak Infrared Aerographic 2424 film. Control plants: solid line; diseased plants: dashed line.

respectively. These three absorbers each remove proportional amounts of blue, green, and red from white light depending on the concentration of the dye deposit. Consequently, the density of a single layer may be measured through a filter, the color of which is complimentary to that of the dye contained in the layer.

Optical density is determined from the ratio of the intensity of the light transmitted through a film to that incident upon it. This ratio, I (transmitted light)/I (incident light) is the transmittance. Its reciprocal is called opacity and the common logarithm of opacity is called optical density. Thus,

$$\text{optical density} = \log_{10} \frac{\text{I (incident light)}}{\text{I (transmitted light)}}$$

The opacity may be due to either silver (in black and white film) or dye material in color film (7).

For this study, optical density measurements were made using an Ansco Model 4 Automatic Recording Microdensitometer. The optical geometry of this type of densitometer records specular density, rather than diffuse density which is usually standard for large-area measurements.

Microdensity scans were made of three positive transparencies made with film 2448, of five positives on film 2443, and of three negatives on film 2424 from the 1971 tests; and of seven positives on film 2443 from the 1972 tests. The microdensitometer aperture slit was 20 × 755 μm with the long dimension oriented parallel to the rows (Fig. 1) and was chosen to integrate random effects such as emulsion grain and plant structure with a total area large enough to separate plant canopy from soil so densities for each would be obtained separately. Each plot image was scanned twice with the centers of the scans approximately one-third the plot width apart.

Drive mechanisms on the recorder were adjusted to produce a chart-to-transparency ratio of 50.8:1. The light beam of the microscope was modified by the introduction of a Wratten No. 29 filter (red) to show only the modulation of the cyan (infrared-sensitive) layer for the 2443 film, similarily, a No. 93 filter (green) was used to record the densities of the magenta (green-sensitive) layer of the 2448 film. Obviously no filter was required for the 2424 film which is neutral in color. The infrared-sensitive layer of film 2443 also records in the same 700-900-nm band as film 2424 with the Wratten No. 89B filter, and these two types of films were used to compare information.

RESULTS AND DISCUSSION.—Positive prints of the three films used are reproduced as Fig. 2. Photographs made on two flight dates, on each of the photographic materials, from the 1971 tests (27 July and 19 August) were chosen to demonstrate changes in crop maturity and disease severity. In this particular plot array only the four panels on each end were evaluated because of the ground conditions mentioned above. Control plots are oriented to the upper left in each case and alternate with diseased plants throughout the random block. Figure 2-A, B show the visible conditions of the plants on the "normal" color film 2448; C and D show high infrared reflection from healthy plants reproduced as red on the false-color film 2443, with reduced red reflection from diseased plants. The black and white infrared records on film 2424, reproduced in the figure as positives, show lighter neutral tones for healthy plants, Fig. 2-E, compared with higher densities (darker tones) for diseased plants, Fig. 2-F.

Original camera-exposed images were measured with the microdensitometer and examples of the traces produced are shown in Fig. 3 for photographs made on the same two dates as those chosen for Fig. 2. Although each plot was scanned twice, only one set of traces (control and diseased) is shown for comparison. Because ground controls were not used, actual densities cannot be compared from one date to another, but density differences between control and diseased plants can be assessed for images contained within the same frame for any particular date.

It has been shown (2), by spectrophotometric measurements, that not only is near-infrared reflectance dependent on refractive index discontinuities of cell wall air-space interfaces in leaves, but also that it is a function of plant maturity. That is, as the plant matures, the leaf mesophyll becomes more spongy with increased air spaces and is, consequently, a more efficient reflector so that the responses (in terms of density in the film) are greater for mature plants than for young plants, but the density differences, across the spectrum, are approximately constant. If assessments are made solely on the basis of film density without reference to crop age, leaves of some stressed plants might show "higher" values than would be obtained from healthy material simply because of cellular compaction from immaturity or deformation from stress.

Although the same plots were photographed on each flight, the differences in the lengths of the traces between dates, and consequently an apparent difference in row spacing, are caused by different scales of the photographs due to slight variations in the altitude of the aircraft (these have not been corrected in the figure). This does not present any difficulty in the computations, because only minimum densities (in the positive transparencies) and maximum densities (in the negatives) were averaged to eliminate possible inter-row shadows or light-toned areas of sunlit soil during early growth when the percentage of the plot area covered by the canopies was small (4). Because the aperture slit on the microscope of the densitometer was wide, for reasons discussed elsewhere, the traces do not conform precisely to the cross-sectional aspect of the canopies of individual rows. As the plants mature, row widths increase, which decreases the density differences between bare soil and foliage in any one photograph, therefore, only densities in the center of the canopies were averaged to determine variation between control and infected plots through the growing season. This is easily done for those readings taken from the 2443 and 2424 films where minima and maxima represent, respectively, the canopy centers, but this simple interpretation cannot be applied to the traces for 2448 film, Fig. 3-A, B, and particularily that shown for 19 August. These represent an apparently erratic result where only 12 rows seem to be recorded, if minima are counted, compared to 13 rows in the other traces and which were present in the plots. As the 2448 film was evaluated with a densitometer using a Wratten No. 93 filter, care must be taken in a visual assessment of the transparencies. In this case, the point of minimum

density; i.e. the maximum of reflected *green* light, is the soil and the maximum densities represent shadow areas. Because these are positives, the minima do not represent row canopy centers as they do for the 2443 film measured through a Wratten No. 29 (Fig. 2-C, D) or as the maxima do (in the negative form) for the black and white 2424 film (Fig. 2-E, F). In other words, the centers of the canopies are at the points of inflection (shoulder of curve) on the traces, which are obvious in the 27 July scan, but less so in the 19 August scan (see arrows Fig. 3, A, B). The inflection points were used to determine average densities for this film.

Although exposures were made by only what appeared to be clear sunlight (i.e., which would produce hard-edged shadows) some high clouds were invariably present. This produced slight exposure inconsistencies which had little effect on the visual evaluations of the photographs, but presumably introduced spurious photometric factors which were reflected in the density traces. Ground control (in the form of targets) was lacking, and therefore values are relative only within individual photographs which precluded comparisons of absolute densities between different dates.

The optical density differences recorded between control and diseased plants for the 27 July flights are slight, which is to be expected because of incipient disease, Table 2. The averages of eight traces each for control and diseased plants show density differences of only 0.02 for the 2448 film, 0.01 for the 2443 film, and 0.08 for the 2424 film, Table 2. (It should be emphasized that a density variation of 0.01, and possibly 0.02, is not significant and can be due to variables in each step throughout the system). By comparison, the traces for the 19 August flights show density differences of 0.01 for the 2448 film; 0.16 for the 2443 film, and 0.29 for the 2424 film. The latter difference would indicate that a greater detection range exists under these particular conditions using monochrome infrared-sensitive films compared with infrared and normal color films, when evaluated through filters transmitting in the regions which record the reflected infrared and green radiation, respectively. The transmission density differences, on the 2424 film, between control and diseased plants was 15 times greater in the 19 August 1971 photographs than in those of 27 July and 5 August on the same film. In each case, the control plots on the 2424 film had the higher numerical values, as was the case with the 2448 film. Because the 2424 film was assessed in *negative* form it is reasonable that higher densities occurred from the healthy canopies, but the same does not hold for the 2448 film which was measured in *positive* form; i.e., higher densities should be from diseased plants because of reduced green reflection. Furthermore, generally higher density differences in the 2424 film were probably due to the higher contrast characteristics of the material. Also, higher density averages for the 2443 film occurred in images of diseased plants with the exception of 5 August, but the difference of 0.01 is so slight that all larger values may be considered to be from diseased plants; i.e., those with reduced infrared reflection.

The averages for the 2443 film in 1972 also showed higher values for diseased plants with the maximum occurring 19 August 1971 and 21 August 1972. Greater differences occur at these two dates because this is when the crop reached maturity, beyond which time the plants became senescent. This effect is indicated by reduced differences for 29 August and 5 September 1972.

Comparing the responses of the infrared-sensitive layer of 2443 film with the 2424 film mentioned above, the greatest density difference in each case, between control and diseased plots, occurs in photographs taken 19 August (Table 2) while for the 2448 film the maximum in reflected green radiation between control and infected plots occurs on 5 August, but the latter difference is too small to be compared with either of the other two films.

Color infrared films also record, from healthy or stressed plants, two bands from the visible region; i.e., green and red (1). Future work will compare data contained in the green band of the normal-color film 2448 (evaluated in these tests), with that contained in the green-sensitive layer of color infrared film 2443, which is the yellow-forming layer, to determine if density differences in either or both films may be used to monitor disease progression. Also, comparisons will be made between the red reflection recordings of 2448 (cyan dye layer) and 2443 (magenta dye layer) films. This will be attempted because it has been reported that density measurements made from color infrared positive transparencies of normal and chlorotic sorghum plants showed significantly higher density readings for normal plants for each sensitivity layer and for the three layers in combination, with the largest numerical difference occurring with green-filter densities (3). In other words, greater density differences were detected in reflected *red* wavelengths because green-filter readings are measurements of the modulation in the magenta dye layer which, in color infrared films, is the red-sensitive layer (1).

Although obvious differences appeared after mid-July between the diseased and healthy plots in both years when plants were assessed for disease by ground truth, density measurements of films recording plant development of healthy and diseased conditions from 5 July to 5 August 1971 were quite similar. In fact, density levels determined from the three films remained fairly constant in both control and infected plots for the period 20-27 July and 5 August (Table 2). Density traces taken from the 12 August and 19 August photographs showed increased differences, particularly for the 2424 film. During the interval from the time of plot inoculation (25 June) until 5 August, a gradual increase in infection was noted. On 5 August, an average of ten leaves per plant were infected, and the area of leaf infection was over 5%. Essentially similar results were shown in 1972, although only 2443 film was tested. Little difference was noted in density levels between healthy and diseased plots on 18 July (0.36 healthy, 0.37 diseased), and none for 27 July (0.38 healthy, 0.38 diseased). On the 2 August 1972 flight and on the four successive flights obvious density differences occurred with the greatest of 0.23 on 21 August.

From the above findings it was concluded that direct measurements of density levels taken from sequential photographs of diseased and healthy field bean plots is not realistic or feasible with the techniques used here in the early stages of disease onset, but in the later stages of the epiphytotic, notable optical density differences are measurable. Although an increasing density difference can be seen for both years for the color infrared 2443 film, the differences contained in any of the three films tested

did not parallel the gradual progression of infection as determined by ground truth assessments throughout the season. Maximum differences occurred with crop maturity and decreased thereafter, although the symptoms continued to intensify. The decrease in density difference is due, probably, to similar reflection qualities from both diseased and healthy plants.

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