FACTORS AFFECTING VIABILITY OF SPORE INOCULUM IN *PERONOSPORA TABACINA* ADAM AND LESION PRODUCTION IN TOBACCO PLANTS

II.* LESION PRODUCTION

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Summary

Inocula containing spores of *P. tabacina* were used as sprays or drops on leaves of tobacco plants to determine the effect of various treatments on lesion production.

The percentage of drops that produced lesions on tobacco leaves decreased from 73 before spores were stored in water to 1 after storage for 24 hr. When spores stored in water at four temperatures were spray-inoculated on to tobacco plants the number of spores required to produce a lesion increased from 1·4 before storage to 90 after storage for 24 hr.

The percentage of drops that produced lesions on plants inoculated with spores stored at 20°C and 43% relative humidity decreased from 55 to less than 1 after 23 days.

The number of lesions produced by inoculation varied with spore viability, inoculation procedure, drop size, leaf treatment, age of plants, variety of tobacco, and strain of the pathogen.

When low spore numbers per millilitre were used in drop-inoculations of washed leaves, at least 66% of all drops containing one spore produced lesions. In spray inoculations, the mean minimum number of spores per lesion produced was 1·2.

I. Introduction

Infectivity and disease occurrence, following inoculation of plants by fungal spores, is often measured as an ED_{50} value i.e. the effective dose for a 50% response (Garrett 1966). This number varies from a high value, when many spores are required, to a low one when the required reaction is obtained with very few spores.

The relation between spore number in inocula and the number of infections or effective disease lesions produced or both was investigated by Rowell and Olien (1957), Petersen (1959), and Davidson and Vaughan (1964), working with relatively large numbers of spores of rust fungi. An increase in spore number was followed by an increase in the number of infections or disease lesions. Van der Plank (1967) used their data to establish mathematically that spores can act independently. Hill (1966), working with conidia of *Peronospora tabacina* Adam, obtained results similar to those mentioned above for rust fungi and also showed that leaf susceptibility could be modified by washing, presumably because of the removal of germination inhibitor (Shepherd and Mandryk 1962, 1963). Heather (1967*a*, 1967*b*), in his reports on deposition, germination, and infection of *Eucalyptus bicostata* Maiden by spores of

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Phaeoseptoria eucalypti (Hansf.) Walker, concluded that leaf characteristics were the major factor influencing field infection.

The experiments reported in this paper were concerned with the effect of viability of spore inocula, spore number, method of inoculation, and leaf characteristics on the number of disease lesions produced when the inoculations were made with spore concentrations likely to occur in natural epidemics of *P. tabacina*.

II. Materials and Methods

(*a*) *Plants*

Similar lots of tobacco plants, cv. Virginia Gold, were grown and inoculated under glasshouse conditions. Any differences in susceptibility to infection that may have been due to soil moisture regimes (Rotem, Cohen, and Spiegel 1967) or other factors were common to plants in each experiment but not necessarily between experiments. Usually two leaves, lying in a horizontal plane and completely exposed vertically, were inoculated. Leaf 1, the lowest leaf, measured approximately 12 by 8 cm and leaf 2 approximately 14 by 9 cm. In the early experiments, and for large plants, leaf area was determined on the basis of length × breadth. More usually, and in all experiments involving spray inoculation, the leaves were traced on paper and areas measured with a planimeter to the nearest cm^2. Leaf treatments were washing or not washing the dorsal or ventral surfaces or both with a water spray equivalent to approximately 30 mm rainfall in a period of 10 min.

After inoculation the plants were placed in a humid atmosphere at a temperature of 15–20°C and returned after approximately 18 hr to the greenhouse with a temperature range of 15–25°C. Lesions were removed from leaves with a cork borer as they appeared on the fifth to ninth day after the plants were inoculated. During the 9-day period of the experiments leaf 1 increased in size by approximately 40% and leaf 2 by 140%.

(*b*) *Inoculum*

Spores of APT1 were produced as described in Part I of this series (Hill 1969) and spores of strain APT2 were produced on cv. S01, a tobacco line resistant to APT1. The preparation of inocula involved removal of spores from leaves, filtering, determination of the spore number with a haemocytometer, dilution of the concentration to the required number of spores per millilitre, and, lastly, their application to the leaves by drop or spray. There was no obvious loss of effectiveness of the inocula if the entire operation was completed in less than an hour but more usually it was completed in 30 min.

In most of the experiments the mean number of spores per drop and per millilitre of inoculum, and the percentage germination, were obtained from counts of spores in sample drops incubated overnight on 2% Difco Bacto agar blocks at 15°C (Shepherd 1962). For spray inoculation experiments the number of spores was of the order of 125 per millilitre.

(*c*) *Spray Inoculation*

Plants on a rotary turntable were subjected to a free-falling mist spray of inoculum for 40–60 sec. In previous experiments (Hill 1966) the weight of inoculum per unit leaf area was determined from the weight of inoculum on sample plants. In the present experiments two opposite sites on the turntable were occupied by plants and the other two by filter papers of diameter approximately equal to that formed by the total length of the midribs on the two opposite leaves being inoculated. The two weighed filter papers were inoculated at the same time as each lot of two plants, and reweighed immediately after inoculation. The amount of inoculum that fell on the two horizontal leaves of known area was of the order of 2·5 g per 1000 cm^2. As the number of spores per millilitre was known from counts on agar blocks, the actual number that fell on the leaf and remained in a film of moisture for approximately 18 hr could be estimated. Results were expressed as mean numbers of spores per lesion produced.

(*d*) *Drop Inoculation*

Inoculum placed on leaves as drops from a pipette (28 per millilitre) or hypodermic syringe needle (140 per millilitre) were removed by filter paper after approximately 18 hr. Results were expressed as percentages of the drop sites where lesions of blue mould disease appeared. In subsequent pages these results will be listed as "percentage of effective drops".

III. Experimental Details and Results

(*a*) *Lesion Production by Spores Stored in Water and in a Desiccator*

An examination of the percentages of spore germinations in inocula placed on agar blocks and the percentages of effective drops of the same inoculum on seedling leaves did not disclose obvious direct relationships. However, both were directly related to length of the storage period of the inocula. In each of the three lots of experiments the percentage germinations and the percentages of effective drops were in turn fitted against the inverse of the length of the storage period (plus an appropriate constant). The direct plot of percentage against time was non-linear and this non-linearity was removed by use of the variable $1/(X+C)$, where X = time and C = constant.

(i) *Drop Inoculation with Spores Stored in Water at 20°C*

Concentrations of 50 spores/ml were made up in 1000 ml distilled water at 20°C in Erlenmeyer flasks and stored at 20°C. In each of three experiments the spore concentration was sampled at 0, 3, 6, and 24 hr for germination tests on agar blocks and for drop inoculation of the dorsal surfaces of leaves of washed seedlings. In each of the three experiments, there were 32 plants inoculated with approximately 2750 drops.

Spore germinations, at the time of the first drop inoculation, were usually 98–100%, decreasing to less than 3% after storage for 24 hr. The percentage of effective drops decreased progressively from a mean of 73·3 to 0·8. Most lesions appeared at 5 days after leaf inoculation, or 6 days when the spores had been stored for long periods.

The following regression equations were obtained:

for germination

$$y = -1\cdot502+294\cdot2/(X+3),$$

and for percentage of effective drops

$$y = -19\cdot45+294\cdot2/(X+3),$$

where X = time in hours. The slopes of these equations were not significantly different, hence the decline in percentage germination with time was matched by a similar decline in the percentage of effective drops.

(ii) *Spray Inoculation with Spores Stored in Water at Four Temperatures*

When spore suspensions of approximately 125 spores per millilitre of water were stored at 5, 15, 20, and 25°C for 0, 3, 6, and 24 hr before being used as spray inoculum for eight plants at each time period, there was an increase in the number of spores

required for the production of a lesion from a mean of 1·4 at 0 hr to 90 at 24 hr. This corresponded with a drop in percentage germination, for all temperatures, from 98·5 to 3%. Germination after storage for 6 hr at 15°C was less than 1%.

For statistical analysis the results for temperatures of 5, 20, and 25°C, being similar, were pooled. Regression equations for the pooled data were:

for germination

$$y = -33\cdot41+1332/(X+10)$$

and for spores per lesion

$$y = -30\cdot31+1001/(X+10).$$

Regression equations for the data at 15°C were:

for germination

$$y = -18\cdot45+115\cdot2/(X+1),$$

and for spores per lesion

$$y = -11\cdot29+73\cdot47/(X+1).$$

The regression equations for the two sets of data were of the same form but the rate of decline was less for spores per lesion than for percentage germination. The rate of decline for both factors was much greater with the more rapid loss of viability at 15°C than for the pooled data of 5, 20, and 25°C.

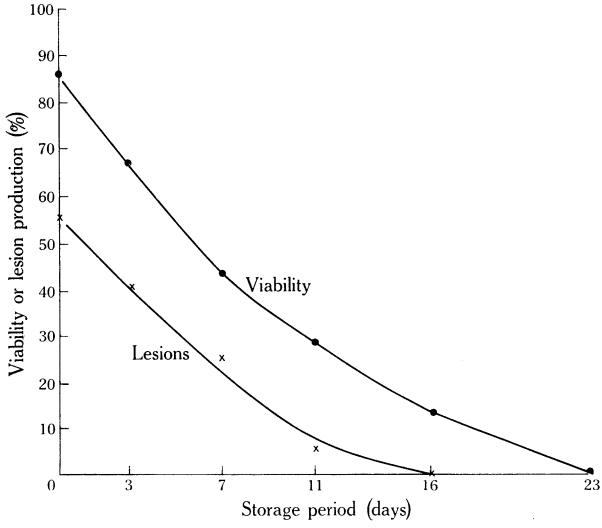

Fig. 1.—Effect on viability (expressed as percentage germination) and lesion production (expressed as percentage effective drops) of storage of spores at 20°C and 43% relative humidity. Means of five observations were plotted in all cases.

(iii) *Drop Inoculation by Spores Stored in a Desiccator*

Spores collected dry by the procedure adopted by Shepherd (unpublished results) and stored in a desiccator (Hill 1969) were sampled at 0, 3, 7, 11, 16, and 23 days and made up into water suspensions containing 50 spores/ml. There were five experiments. At each time period two leaves of eight plants were inoculated with approximately 650 drops of spore suspension.

At zero time the mean percentage of effective drops was approximately 30% below the mean percentage germination on agar blocks (Fig. 1). This difference

decreased with storage time and decrease in spore viability. The calculated ED_{50} value (mean of 1·786 spores per drop) occurred at 2 days storage. At day 23 there was no germination on agar blocks in any of the five experiments. Nevertheless, lesions appeared on less than 0·5% of the infection sites. Uninoculated plants remained healthy.

Regression equations were:

for germination

$$y = -57 \cdot 17 + 2203/(X+15),$$

and for percentage of effective drops

$$y = -44 \cdot 67 + 1493/(X+15),$$

where X = time in days. The slopes were significantly different at the 5% level.

(*b*) *Effect of Washing Dorsal or Ventral Leaf Surfaces on Lesion Production by Drop or Spray Inoculation*

(i) *Drop Inoculation*

There were three experiments, each containing eight plants. Approximately 400 drops of inoculum were placed on two leaves of four plants for each pair of the treatments, dorsal and ventral leaf surfaces being washed or not washed in all combinations. The spore suspensions contained 50 spores/ml and the pipette delivered 28 drops/ml.

TABLE 1

DROP INOCULATION OF TOBACCO PLANTS: COMPARISON OF MEAN PERCENTAGE OF EFFECTIVE DROPS IN VARIOUS TREATMENTS

D.S., dorsal leaf surfaces. V.S., ventral leaf surfaces. S.D., standard deviation

Treatments	Difference	S.D. of Difference	*t*-test
D.S. washed *v.* D.S. not washed	22·6	5·10	4·43***
D.S. washed *v.* V.S. washed	−1·8	6·15	−0·29†
D.S. washed *v.* V.S. not washed	15·1	4·80	3·15**
D.S. not washed *v.* V.S. washed	−24·4	6·32	−3·86***
D.S. not washed *v.* V.S. not washed	−7·5	5·02	−1·49†
V.S. washed *v.* V.S. not washed	16·9	6·08	2·78**

** Significant at the 1% level.
*** Significant at the 0·1% level.
† Not significant.

The results from the statistical analysis of the data are shown in Table 1. The mean percentage of effective drops for washed dorsal leaf surfaces, unwashed dorsal leaf surfaces, washed ventral leaf surfaces, and unwashed ventral leaf surfaces were 73·0, 50·4, 74·8, and 57·9 respectively. Clearly, with drop inoculation there was a significant increase in the susceptibility of both dorsal and ventral leaf surfaces as a result of washing.

(ii) *Drop Size*

The effect of drop size on the percentage of effective drops was investigated by diluting a spore suspension to provide two spore concentrations, one for use with a pipette delivering 28 drops/ml and the other to provide the same number of spores/drop from a hypodermic syringe needle delivering 140 drops/ml. In three experiments the spore number per drop for pipette and hypodermic syringe needle differed by $\pm 0 \cdot 1$ spores from the means of $1 \cdot 85$, $3 \cdot 1$, and $3 \cdot 2$. In the fourth experiment the variation was $\pm 1 \cdot 25$ in a mean of $9 \cdot 35$ spores per drop. In these experiments a total of 5140 drops were placed on leaves of 116 plants. All drops remained on the leaves for the same time period.

The four experiments were treated as blocks in the analysis of variance for the two factors, leaves washed *v.* not washed and drops from the pipette *v.* those from the hypodermic syringe needle. As in other drop-inoculation experiments, there was a significantly greater number of lesions on washed leaves than on unwashed leaves where pipette drops were used. There was no significant difference between washed and unwashed leaves where the hypodermic syringe needle was used. The percentages of effective drops for both pipette and hypodermic needle are set out in the following tabulation as means of each experiment:

Experiment	1	2	3	4
Percentage of effective drops				
Needle	15·8	5·3	3·0	6·2
Pipette	25·2	24·3	6·0	31·2

The analysis showed that the percentage of effective drops delivered by the hypodermic syringe needle was significantly less ($0 \cdot 1\%$ level) than that delivered by the pipette.

(iii) *Spray Inoculation*

The series of experiments to determine the effect of leaf washing on the susceptibility of dorsal and ventral surfaces to inoculation by drops was repeated using spray inoculation. There were six experiments in each comparison of washed and unwashed dorsal and ventral leaf surfaces, and eight plants per experiment. The analysis of the results (Table 2) followed the same lines as that for drop inoculation, but was performed on the mean number of spores per lesion. The mean number of spores per lesion for washed dorsal leaf surfaces, unwashed dorsal leaf surfaces, washed ventral leaf surfaces, and unwashed ventral leaf surfaces were $1 \cdot 84$, $2 \cdot 53$, $2 \cdot 15$, and $2 \cdot 68$ respectively. Clearly, washing the dorsal leaf surface significantly increases susceptibility because fewer spores are required for the production of a lesion but, unlike drop inoculation, washing the ventral leaf surface did not increase its susceptibility significantly.

(*c*) *Lesion Production by Spores Removed from a Leaf at 8 a.m. and at 8, 16, and 24 hr Thereafter*

Spores were removed at 8-hourly intervals from one-fourth of a leaf uniformly covered with dense sporulation. Spores removed at each of the four time periods were used to spray-inoculate the dorsal surfaces of four plants washed and dried with

filter paper and four plants washed and not dried. For 9 of the 12 inoculations involved in three experiments, there were also two plants not washed. The washed plants were thus in two groups to determine the necessity for drying leaves of plants prior to mist-spray inoculation.

The data were examined as a split-plot randomized block with experiments as blocks, time periods of inoculation as main treatments, and the three leaf treatments (washed and dried, washed and not dried, and not washed) as subtreatments, followed by the logarithmic transformation to stabilize the variance.

TABLE 2

SPRAY INOCULATION OF TOBACCO PLANTS: COMPARISON OF MEAN NUMBER OF SPORES PER LESION IN VARIOUS TREATMENTS

D.S., dorsal leaf surfaces. V.S., ventral leaf surfaces. S.D., standard deviation

Treatments	Difference	S.D. of Difference	*t*-test
D.S. washed *v.* D.S. not washed	−0·643	0·187	−3·44**
D.S. washed *v.* V.S. washed	−0·310	0·233	−1·33†
D.S. washed *v.* V.S. not washed	−0·833	0·257	−3·24**
D.S. not washed *v.* V.S. washed	0·373	0·257	1·45†
D.S. not washed *v.* V.S. not washed	−0·150	0·279	−0·54†
V.S. washed *v.* V.S. not washed	−0·523	0·308	−1·70†

** Significant at the 1% level. † Not significant.

There were no significant differences in the germinability of spores collected from the same leaf (see also Shepherd 1962) or in the number of spores per lesion produced for each of the four time periods of inoculation. Nor was there a significant difference between leaves washed and dried, and leaves washed but not dried. Here, as in other experiments, washed dorsal leaf surfaces were more susceptible than unwashed, as shown by the following tabulation:

Leaf Treatments	Transformed Means Derived from Lesion Production
Washed and dried	0·675
Washed and not dried	0·711
Not washed	0·917

Least significant differences were 0·165 at the 5% level and 0·230 at the 1% level.

(*d*) *Persistence of Susceptibility of Washed Leaves*

There were four experiments in each of which four plants were washed at each of 48, 24, and 0 hr before inoculation and four plants were not washed. Two leaves of each plant were drop-inoculated with a spore suspension containing 50 spores/ml. There were approximately 300 drops on leaves of each group of four plants. Treatment means for effective drops, derived from an analysis of variance, were as follows:

Leaves not washed	34·7	Leaves washed 24 hr	44·5
Leaves washed 48 hr	41·4	Leaves washed 0 hr	63·5

The least significant difference for comparing treatment means (at the 5% level) is 7·53.

Apparently much of the increased leaf susceptibility due to washing is lost within 24 hr but there still remains an increased susceptibility up to 48 hr after washing.

(e) *Lesion Production on Large Plants: Effects of Washing Leaves, Position of Leaf on the Stalk, and Plant Age*

Tobacco plants, cv. Virginia Gold, grown in large pots in the glasshouse were drop-inoculated on the lower 8–13 leaves when plants were 35–195 cm in height. Tall plants were reduced in height to 105 cm, the maximum height that could be accommodated in the inoculation chamber. Leaf length and breadth and plant height were recorded at the time of inoculation and at the conclusion of the experiment 9 days later. The lowest leaf (leaf 1) measured approximately 31 by 21 cm and the largest leaf about 59 by 36 cm. Each experiment contained two plants and there were 21 experiments.

TABLE 3

MEAN NUMBER OF LESIONS ON WASHED AND UNWASHED MATURE AND IMMATURE TOBACCO PLANTS

Plants were 35–195 cm in height. The lowest 8–13 leaves of 21 pairs of plants were each inoculated with 20 drops of APT1 spore suspension

Leaf No.	No. of Leaves Inoculated	Mean No. of Lesions		No. of Leaves Inoculated	Mean No. of Lesions	
		Washed Leaves	Unwashed Leaves		Washed Leaves	Unwashed Leaves
	Immature plants			Mature plants		
1 (lowest)	17	11·0	9·5	4	2·8	2·3
2	17	9·0	7·2	4	3·0	3·5
3	17	9·3	5·2	4	2·5	0·3
4	17	9·0	4·1	4	1·8	1·3
5	17	9·7	3·5	4	4·8	1·3
6	17	10·0	3·1	4	0·3	0·5
7	17	9·4	2·5	4	3·5	0·8
8	17	8·6	2·1	4	4·8	0·8
9	14	8·6	1·1	4	3·0	0·3
10	14	5·9	1·0	4	1·3	0·8
11	11	5·9	0·6	4	1·0	0·3
12	11	6·5	0·2	4	1·0	0·3
13	11	5·3	0·2	4	0·7	0

Twenty separate drops from a spore suspension containing 50 spores/ml were placed in a defined area on the upper surface of each leaf. The number of lesions per washed leaf of immature plants was of the order of six to nine, and for mature plants (inoculated several weeks after flower heads were removed) two to three. Unwashed leaves had less than half those numbers respectively (Table 3).

For each leaf the effect of age and washing was examined by an analysis of variance. This showed that washed leaves had significantly more lesions (0·1% level) than unwashed leaves except for the two basal leaves. It also showed that immature plants had significantly more lesions (0·1% level) than mature plants.

Linear regressions of the number of lesions against each leaf position on the stalk (reciprocal leaf number for unwashed leaves) were calculated for both washed and unwashed leaves separately. Whilst only two out of five regressions (five groups of plants by height, four immature and one mature) were significant for washed plants, and only one highly so, all five regressions were highly significant for unwashed plants. Thus the reduction in the number of lesions per leaf increases more rapidly with increasing height for unwashed than for washed plants.

Simple correlations of the number of lesions per leaf with the percentage change in leaf area were computed for the plants in the five height groups and for washed and unwashed plants separately. Correlations were also obtained for washed and unwashed leaves combined over ages of plants and finally an overall correlation combined over all data. Only five of the ten correlations were significant (mostly at the 5% level) but all the pooled correlations were highly significant at the 0·1% level. This evidence showed that since the correlations are all negative, the greater the change in leaf area the less the number of lesions. Thus, the young unexpanded leaves of immature plants were less susceptible to infection than the older leaves on the same plant.

(*f*) *Minimum Spore Number per Lesion*

In seven experiments the mean number of spores per drop in 632 sample drops was $1 \cdot 1 \pm 0 \cdot 2$. Of these drops 27·5% had more than one spore and 40% had one spore. Thus, the total possible number of lesions was 67·5% of the total drops of inoculum on the leaves. There were 2017 drops on leaves and of these, 27·5% or 554·7 contained more than one spore. If we assume that lesions occurred at all drop sites where there were more than one spore, then the 536·3 remaining lesions (1091 − 554·7) were caused by infection by one spore. This means that 66·5% of all drops containing one spore produced lesions.

In spray-inoculation experiments, inoculum density and quantity per leaf were adjusted to avoid any possibility of confusion due to the occurrence of an excessive number of lesions. There were no experiments on minimum number of spores per lesion but in the course of the spray-inoculation experiments reported herein, the mean minimum number of spores per lesion was 1·2 on 11 occasions.

(*g*) *Comparative Susceptibility to* P. tabacina *Strain APT2 of Two Tobacco Lines Resistant to APT1, and the Susceptible Cultivar Virginia Gold*

Inoculum containing 50 spores/ml was used as drops from a pipette delivering 28 drops/ml.

In these experiments the washed and unwashed dorsal leaf surfaces of plants of the cultivars S01, S01 selection, and Virginia Gold were inoculated, with results as shown in Table 4.

A comparison of results for the three varieties showed that S01 was slightly more susceptible than Virginia Gold and significantly more susceptible than S01 selection, particularly in leaf 1 and in unwashed leaves. There was a greater difference in susceptibility between leaves 1 and 2 of unwashed Virginia Gold plants than in similar plants inoculated with spores of APT1.

For all varieties, the percentage of effective drops was much lower than would be expected for APT1 spores on Virginia Gold plants.

TABLE 4

TOTAL AND EFFECTIVE NUMBERS OF DROPS OF INOCULUM CONTAINING SPORES OF APT2 ON TOBACCO LINES RESISTANT AND SUSCEPTIBLE TO APT1

The cultivars S01 and S01 selection are resistant and Virginia Gold susceptible to APT1

Cultivar	Treatment*	Leaf No.	No. of Drops of Inoculum	Effective Drops (%)	No. of Plants	Total No. of Drops	Mean No. of Effective Drops (%)
S01	D.S. W	1	1033	38·3	34	2608	37·7
		2	1575	37·2			
	D.S. N	1	143	11·2	6	376	6·9
		2	233	4·3			
S01 selection	D.S. W	1	712	1·0	24	1756	17·2
		2	1044	22·5			
	D.S. N	1	402	1·0	12	882	1·0
		2	480	1·0			
Virginia Gold	D.S. W	1	1241	33·2	38	2907	31·5
		2	1666	30·2			
	D.S. N	1	158	17·7	6	400	10·8
		2	242	6·2			

* D.S. W, dorsal surface washed; D.S. N, dorsal surface not washed.

IV. DISCUSSION

Spore production in *P. tabacina* was investigated by Cruickshank (1961*a*, 1961*b*, 1963), Rider, Cruickshank, and Bradley (1961), and Shepherd and Mandryk (1964); spore survival by Hill (1962), Krober (1965), Bromfield and Schmitt (1967), Jankowski (1967), and others; germination *in vitro* by Cruickshank (1961*a*) and Shepherd (1962), and *in vivo* by Shepherd and Mandryk (1963). Although single spore transfers to leaves have resulted in lesions (Shepherd, Stuart, and Mandryk 1963) very little has been done to relate spore viability and spore number to lesion production.

Conditions for spore production, as shown by the normal 98–100% capacity for germination at the time of harvesting, appeared to be satisfactory, but differences in the rate of loss of viability under stress (Hill 1969) could be indicative of varying capacities for establishment of infection as measured by lesion production. This and other undetermined factors limited the effectiveness of single spore inoculations to a mean of 66% of the possible number. In some experiments with both drops and sprays there was a higher percentage. In general, the decline in the percentage of effective spores was matched by the decline in percentage spore germination.

(*a*) *Lesion Production on Leaves of Small Plants*

It was shown previously (Hill 1966) and confirmed in the field (Paddick 1965) that the dorsal surfaces of washed leaves of tobacco plants were more susceptible to infection by *P. tabacina* than unwashed leaves. This increased susceptibility of the dorsal surface occurred with both drop and spray inoculations and for the ventral surface with drop inoculation only (Tables 1 and 2).

The expectation was that ventral leaf surfaces, because of their mesophyll structure, high ambient humidity, fewer leaf hairs (Bentley and Wolf 1945), and approximately three times as many stomata (Barnard 1960), would be much more susceptible to infection than dorsal surfaces. Rotem, Cohen, and Spiegel (1967) did not find any relationship between incidence of *P. tabacina* and number of stomata, nor is there any evidence from the present experiments to indicate that the ventral surface is more susceptible to infection than the dorsal surface.

Bjorling and Sellgren (1955) studied the incidence of infection by potato late blight on the upper and lower surfaces of leaves of potato plants in the field. They found ten times as many sporangia lodged on the upper surface compared to the lower and seven to eight infections were established on the upper surface for one on the lower. These results, and those for *P. tabacina* on tobacco, showed the necessity for adequate protection of dorsal leaf surfaces by fungicides.

The relatively poor results obtained with drops of inoculum from a hypodermic syringe needle were unexpected and require further investigation. Differences due to drop size were also reported by Lapwood and McKee (1966) for inoculum of *P. infestans*.

In a saturated atmosphere spores remain attached to the conidiophores (Hill 1961), but viability (see also Hill 1969) and capacity for lesion production are not affected for 24 hr or longer. Thus, the number of spores available for dissemination during periods of persistent high humidity could increase daily, to provide formidable difficulties in disease control when the total spore mass is disseminated. This is a continuing possibility in infected seedbeds and an occasional occurrence in the field.

(*b*) *Lesion Production on Leaves of Large Plants*

The susceptibility of the dorsal surface of leaves of large plants was greatly increased by washing except for the two lowest leaves which were equally susceptible as washed or unwashed leaves. Fewer lesions and smaller differences occurred in leaves of mature topped plants. Washing increased leaf susceptibility in all leaves, whereas with unwashed plants it decreased rapidly with increasing height of leaves on the stem (Table 3). For both treatments the relatively small unexpanded leaves toward the top of the plant were least susceptible. When these results were considered in conjunction with the great reduction in susceptibility at 24 hr after washing, it would appear that, provided the bottom leaves have protection against infection, increased vulnerability due to rain or spray irrigation would be lost in a little more than 24 hr of fine weather.

(*c*) *Lesion Production by Spores of Strain APT2*

The cultivar Virginia Gold was not as susceptible to APT2 as it was to APT1 (Table 4), and this result may be compared with that of Shepherd and Mandryk

(1967) on the relative intensity of their necrotrophic reaction on leaf disks at 15°C. There was a relatively low percentage of effective drops and a wider difference in susceptibility between washed and unwashed leaves. Lesions tended to be more localized and the overwintering of the pathogen in the stem as a result of infection spreading from isolated lesions was less likely to occur. Mycelial spread through leaf tissues was more rapid with cv. S01.

The other resistant line, a selection from S01, was less susceptible to APT2 than S01, reacted differently in the two leaves inoculated, and the unwashed leaves were very resistant. These differences could be due to APT2 being selective for S01, on which the spores were produced, or alternatively the factors for resistance were not the same in the two cultivars.

(*d*) *Number of Spores per Lesion*

The success with the establishment of lesions by one spore, as could be expected in the field, was considered due largely to the production of spores with near 100% germination at the time of inoculation. Many reports (*loc. cit.*) stress the need for large numbers of spores in order to assure infection; others stress environmental factors, host plant physiology, or the need for additives to inocula. Spore viability and the direct modification of host plant susceptibility, as occurs in the field, have received attention in this paper.

In a study of spore numbers in lesion production, Lapwood and McKee (1966) did not give figures for spore viability. Detached potato leaflets were inoculated with drops containing zoospores of *P. infestans* in a water suspension supplemented with tuber extract. The ED_{50} value from probit analysis ranged from 6 to 15 zoospores per drop for varieties with little or intermediate field resistance. The sporangia of Bjorling and Sellgren (1955) germinated poorly but their effectiveness may have been due to zoospores formed. For *P. infestans* it would appear that deposition of sporangia following aerial dissemination could be followed by formation of zoospores subject to splash distribution and individually capable of lesion production.

(*e*) *Inhibitors of Germination*

In the experiments reported herein the rapid loss of viability in the prepared inoculum was probably associated with leaching of nutrient from the spores and the increased susceptibility of leaves was due to the removal of an inhibitor present on the leaf surface, particularly the dorsal surface. Shepherd and Mandryk (1963) obtained estimates of the amount of germination inhibitor present on unwashed leaves, and correlated this with leaf susceptibility data reported by Hill (1959). Martin, Blatt, and Burchil (1957) extracted substances from ether washings of apple leaves that inhibited germination of spores of *Podosphaera leucotricha*. Holomon (1967) suggested that the phylloplane flora on potato leaves may act as a barrier to establishment of infection by zoospores of *Phytophthora infestans*. The removal of external barriers to infection is probably a prerequisite to an epiphytotic and their recognition is essential to a successful programme for disease control. However, inhibitors to infection were not a factor in the two lowest and oldest leaves of large tobacco plants. With spore germination about 100%, and maximum susceptibility in the host plant,

the infective capacity of the inoculum can be determined. However, these conditions are seldom attained and the number of infections is limited by unfavourable environmental conditions.

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VI. References

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