GERMINATION OF CONIDIA OF *PERONOSPORA TABACINA* ADAM

I. GERMINATION IN VITRO

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Summary

Washed conidia of *Peronospora tabacina* Adam germinated poorly or not at all in water alone, but germinated in the presence of riboflavin. The rate of germination of conidia in liquid suspension was enhanced by the presence of carbon and nitrogen sources, phosphate, and calcium and magnesium ions. The effects of 141 metabolites on germination and germ-tube elongation have been tested.

A number of analogues of natural metabolites were inhibitory to germination and germ-tube elongation. Extracts and exudates of tobacco leaves did not affect either the amount or rate of germination.

Washing by centrifugation increased the percentage of conidia that germinated subsequently and the presence of a germination inhibitor in unwashed conidia is postulated.

The optimum temperature for germination of conidia on agar, or in liquid suspension, was in the range 15–20°C. The pH optimum varied with the constitution of the medium; when germinated on 2% agar a broad optimum was shown at pH's 5·5–8·0, whereas in liquid suspension the optimum was in the pH range 6·5–8·0.

When conidia were tested within 24 hr from the time of initiation of sporulation, germination was high (85%). After 48 and 72 hr germination had dropped to 61 and 48% respectively.

Washed spores showed no loss of germination capacity when kept for up to 6·5 hr, but germination was negligible after 18 hr storage.

Visible light did not affect germination, but 9000 μW/cm^2 of ultraviolet light reduced it to 2·7%.

I. INTRODUCTION

Angell and Hill (1931*a*, 1931*b*) reported that the conidia of *Peronospora tabacina* Adam would germinate in water on glass slides, but noted that extreme variability occurred in the viability of freshly gathered conidia. Wolf *et al.* (1934), Armstrong and Sumner (1935), and Clayton and Gaines (1945), using the same technique, also reported considerable variability in germination.

Angell and Hill (1932) demonstrated that maximum germination occurred in 2 or more hours at 12–24°C, that germination was slower outside this range, and was completely inhibited below 0°C and above 28°C. Wolf *et al.* (1934) reported that germination occurred within 2 hr at 4–18°C and Armstrong and Sumner (1935) reported optimum germination over the range 15–23°C. However, Clayton and Gaines (1945) stated that the optimum temperatures for germination ranged from 1·5–26·1°C for different conidial collections, while Cruickshank (1961) reported an

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optimum range of 15·2–27·2°C for germination after incubation for 6 hr, and a range of 8·6–27·2°C after incubation for 12 hr.

Armstrong and Sumner (1935) reported that viable conidia could be collected at all hours of the day between 8 a.m. and 5 p.m., but Clayton and Gaines (1945) stated that the time of collection of conidia influenced the percentage germination attainable, maximum values occurring with conidia collected at 6 a.m., and lower values with conidia collected at 11 a.m.

Wolf *et al.* (1934) demonstrated that germination was high over the pH range 3·6–7·0 and that it was lower outside this range.

Angell and Hill (1932) stated that the addition of tobacco leaf extracts or sugar solutions produced no detectable effects on germination, but Cruickshank (1961) reported a stimulating effect on germination of exudates from tobacco leaves, and the results of the latter author indicated that germination was much less variable when conidia were germinated in the presence of complex nutrient materials.

Clayton and Gaines (1945) found that conidia produced on young seedlings (3–4 weeks old) generally showed lower germination percentages than those produced on plants 6–8 weeks old. Maximum germination percentages were found in conidia produced at a day temperature of 25°C and a night temperature of 15°C, and minimum germination percentages in conidia produced at 10°C continuously. Armstrong and Sumner (1935) found that pale-coloured spores, produced in humidity cabinets, showed very low germination percentages.

It is the aim of the present paper to define the environmental factors affecting germination of conidia of *P. tabacina* under reproducible physical and chemical conditions. Cultural conditions are described that allow high and reproducible levels of germination. Previous work by other authors is discussed in the light of the findings reported herein, that unwashed conidia contain an autoinhibitor of germination, and that washed conidia exhibit a riboflavin requirement for germination.

II. Materials and Methods

(*a*) *Conidial Production*

Nicotiana tabacum cv. Virginia Gold plants were grown in 6-in. flower pots in soil mix *C* supplemented with fertilizer IIc, as described by Matkin and Chandler (1957). When the plants were approximately 80 cm high, the foliage was inoculated with a conidial suspension of *P. tabacina* in water and placed under conditions favourable for leaf infection (Cruickshank 1958). After 7 days, sporulation was induced by placing the plants overnight at a temperature of 20°C and a relative humidity approaching saturation.

The "Canberra" ecotype of *P. tabacina*, which was originally isolated in Victoria, was used in all experiments.

Conidial suspensions were prepared from entire leaves harvested within 24 hr of the induction of sporulation, the conidia being removed by immersion of the leaves in distilled water. Fragments of conidiophores were removed by filtration through a loose cotton-wool plug, and the conidia washed twice by centrifugation and then

resuspended in distilled water. The density of the suspension was adjusted to 10^5 conidia/ml.

For the estimation of suspension densities a standard curve was constructed by plotting the number of conidia per millilitre of suspension, as determined by haemocytometer counts, against the optical density at 440 mμ in a Unicam SP600 spectrophotometer. The curve was linear over the range $2–30 \times 10^5$ conidia/ml.

(*b*) *Germination on Agar—Basic Method*

A suspension (0·05 ml) containing 5×10^3 conidia was placed on a block of 2% Difco Bacto agar of dimensions 15 by 15 by 3 mm resting on a microscope slide in a petri dish, the lid and base of which were lined with moistened filter paper. Water (0·05 ml), or the appropriate solution, was immediately added to the conidial suspension on the block, which was then incubated for 4 hr at 15°C. At the end of the incubation period one drop of formalin was added to the suspension. The percentage germination was determined by counting the number of conidia that showed a recognizable germ tube. One hundred conidia were examined on each block, and each experimental treatment was replicated 10 times. The mean germination of controls (30 replicates) was $90 \cdot 9 \pm 5 \cdot 85\%$.

(*c*) *Germination in Liquid Suspension—Basic Method*

Washed conidia were suspended in a basal medium of the following composition:

Sorbitol	1·2 g
Riboflavin	0·003 g
Magnesium sulphate ($MgSO_4.7H_2O$)	0·24 g
Disodium hydrogen phosphate (Na_2HPO_4)	0·5 g
Calcium chloride ($CaCl_2$)	0·24 g
Potassium nitrate (KNO_3)	0·24 g
Water	1200 ml

Initial pH adjusted to 7·5

100 ml suspension, containing $10^7–10^8$ conidia, was placed in a vertical glass tube of dimensions 50 by 2·5 cm and furnished with a jet orifice, 0·4 cm in diameter, at the bottom and covered loosely at the top with a flat-bottomed glass vial of dimensions 3 by 7 cm. Sterile air at the rate of 3 litres per minute was bubbled through the suspension, which was incubated at 15°C. Twelve such tubes were used concurrently. At appropriate intervals, 1-ml samples of the conidial suspension were withdrawn, added to 0·05 ml formalin, and germination determined as in Section II(*b*).

(*d*) *Estimation of Germ-tube Length*

Germ-tube length was determined with the aid of a microscope ocular micrometer scale calibrated in microns (1 division = 4·1 μ). Thirty germ tubes were measured in each experimental treatment.

After incubation for 4 hr at 15°C on agar, the mean germ-tube length was $38 \cdot 7 \pm 7 \cdot 8$ μ, while in liquid suspension under similar conditions the mean germ-tube length was $37 \cdot 3 \pm 9 \cdot 3$ μ. The mean germinations recorded were 94·3 and 92·7% respectively.

(e) *Chemicals and Apparatus*

All reagents used were A.R. grade. All sugars, vitamins, and amino acids were obtained from Messrs. L. Light & Co. Ltd., Colnbrook, England, and all purines and pyrimidines from Nutritional Biochemicals Corporation, Cleveland, U.S.A.

A Jones model B electrometer was used for pH measurement. A Hanovia low pressure quartz ultraviolet lamp was used for ultraviolet irradiation.

III. Experimental and Results

(a) *Effect of Conidial Concentration and Washing on Degree of Germination*

With conidial concentrations in excess of 10^6/ml the percentage germination was virtually nil on 2% Difco Bacto agar, but it rose to maximum values as the conidial

Table 1

Effect of substrate composition on germination
Incubation was for 4 hr at 15°C

Substrate Composition	Mean Germination (%)
Glass	0·6
2% Difco Bacto agar	88·0
2% gelatin (BDH Gold Label)	73·6
2% pectate gel*	59·8

* Prepared according to Rudd-Jones (1946).

concentration was lowered to 5×10^4/ml. This fact suggested the possible presence of a germination inhibitor and this hypothesis was supported by a study of the effects of washing on subsequent germination.

Conidia were removed from leaves by washing and the suspension density was adjusted to 5×10^5/ml. These gave a mean germination of 57·2% (range 44–72%). A similar conidial suspension, washed once by centrifugation in distilled water for 15 min, gave a mean germination of 85·1% (range 66–92%). After two washings, a similar sample showed a mean germination of 90·0% (range 83–99%).

The supernatant from the original conidial suspension in the above experiment, when added to twice-washed conidia, reduced the mean germination to 64·3%. When 25 ml of the original supernatant was lyophilized and the residue dissolved in 1 ml of water, addition of the solution to twice-washed conidia completely suppressed germination, indicating the presence of a water-soluble inhibitor in the unwashed conidia of *P. tabacina*. The isolation and properties of this material have been described by Shepherd and Mandryk (1962).

Twice-washed conidia were used in all experiments reported below.

(*b*) *Effect of Substrate Composition on Germination on Solid Media*

The reports of previous authors (Angell and Hill 1931*a*; Wolf *et al.* 1934; Armstrong and Sumner 1935; Clayton and Gaines 1945) on the great variability of germination in water on glass slides have been confirmed in the present study, values ranging between 0 and 71% being recorded and great variation being observed between replicates in many cases. In contrast, germination on 2% Difco Bacto agar was universally high, with little variation between replicates. This suggested that the substrate composition might play a part in determining the amount of germination attainable.

TABLE 2

EFFECT OF AGAR CONCENTRATION ON GERMINATION

Concentration of Difco Bacto Agar (%)	Mean Germination (%)	Concentration of Difco Bacto Agar (%)	Mean Germination (%)
0·2	0·6	1·0	83·6
0·4	26·0	2·0	88·2
0·6	37·2	4·0	86·4
0·8	53·7	8·0	83·7

TABLE 3

EFFECT OF AGAR TYPE ON GERMINATION

Agar Type	Mean Germination (%)	Agar Type	Mean Germination (%)
2% Difco Bacto	94·7	2% Oxoid Ionagar No. 2	63·6
2% Oxoid NZ	89·0	2% Difco Bacto Noble	29·4
2% Difco prune	72·1	2% "washed" agar*	2·3

* Difco Bacto agar washed according to the method of Ryan, Beadle, and Tatum (1943).

When conidia were incubated on various substrates, using the basic agar method, germination was high on all solid organic substrates tested, but low in the absence of such materials (Table 1).

However, the degree of germination observed was modified by the concentration of agar, when this was used as the substrate. Table 2 shows the effect of concentration of Difco Bacto agar upon the amount of germination after 4 hr incubation at 15°C. The type of agar used also influenced the amount of germination (Table 3).

Thus, if the concentration of substrate material (e.g. agar) is suitable, germination occurs in the presence of many organic substrates. However, it may be observed

from Table 3 that the more purified types of agar give considerably less germination than those of a lower degree of purity. These observations suggested the presence of a growth factor requirement for germination.

Using the basic agar method, 0·05 ml conidial suspension was placed on "washed" agar and 0·05 ml solution containing growth factor was added. Table 4

TABLE 4

EFFECT ON GERMINATION OF ADDITION OF VITAMIN SOLUTIONS TO CONIDIA ON "WASHED" AGAR
Vitamins added at a final concentration of 20 μg/ml

Vitamin Added	Mean Germination (%)	Vitamin Added	Mean Germination (%)
Nicotinic acid	3·6	Pantothenic acid	9·6
Biotin	9·4	Choline	8·7
Thiamine	15·4	Water (control)	2·8
Pyridoxin	17·6	2% Difco Bacto agar (control)	89·5
Riboflavin	72·0	2% "washed" Difco Bacto agar (control)	2·3

shows the effects of additions of various vitamins, at a final concentration of 20 μg/ml, on germination after incubation for 4 hr at 15°C.

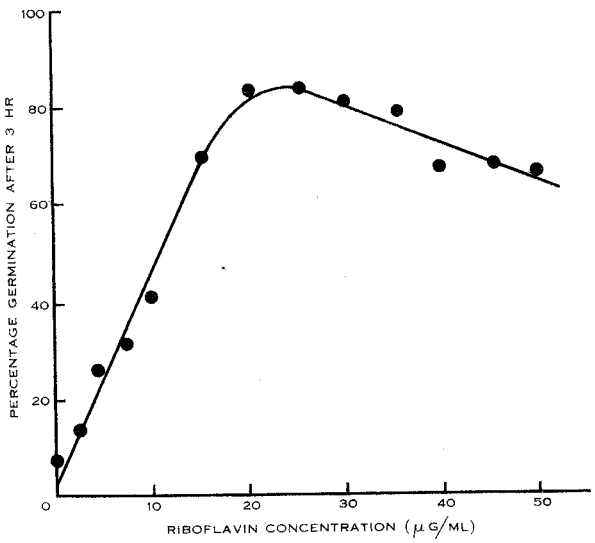

Fig. 1.—Effect of riboflavin concentration on germination in liquid suspension.

Riboflavin was the only vitamin which showed a marked stimulation of germination on washed agar. The effects of various concentrations of riboflavin were tested by the liquid suspension method, the results being shown in Figure 1.

In confirmatory experiments, the analogue isoriboflavin, at a concentration of 20 μg/ml, gave a mean germination of 56·4%, while a mixture of riboflavin and isoriboflavin (10 μg/ml of each) gave a mean germination of 47·0% in a similar test. Samples of conidia used in the above experiment, when incubated on 2% Difco Bacto agar, gave a mean germination of 86·5%; when incubated on glass in the absence of riboflavin, the mean germination recorded was 4·2%, and when incubated on glass in the presence of 20 μg/ml riboflavin the mean germination value was 71·2%. However, in this last case germ-tube growth was restricted, compared with that

TABLE 5

EFFECT OF VITAMINS ON GERMINATION AND GERM-TUBE LENGTH

Compound	Agar Medium*		Liquid Suspension†	
	Mean Germination (%)	Mean Germ-tube Length (μ)	Mean Germination (%)	Mean Germ-tube Length (μ)
Biotin	84·0	26·1	90	35·6
Riboflavin	90·0	32·2	84	31·6
Pantothenic acid	81·5	28·0	86	33·6
Pyridoxin	94·5	38·5	92	28·8
Thiamine	91·5	28·0	84	31·6
Nicotinic acid	94·0	34·3	84	30·4
Choline	91·0	32·2	94	32·8
Water (control)	94·0	40·6	—	—
L.S.D. (1%)	8·6	4·6	8·4	4·1

* Incubation for 4 hr at 15°C. † Incubation for 2 hr at 15°C.

occurring on agar, or in liquid suspension. Using 2% washed agar as the substrate, addition of various amounts of riboflavin gave a similar result to that reported above for germination in liquid suspension. Addition of riboflavin at a concentration of 20 μg/ml to 2% Difco Bacto agar had no significant effect on germination. 140 other metabolites had no significant stimulatory effects on germination on washed agar.

Thus the conidia of *P. tabacina* show a requirement for riboflavin for germination. The particular batch of Difco Bacto agar, when used at a concentration of 2% apparently contained enough riboflavin as an impurity to satisfy the requirements of conidia for germination. In all subsequent experiments in which the basic agar technique was used, 2% Difco Bacto agar was used, and no riboflavin was added.

Further evidence for a riboflavin requirement was obtained by testing the effect of 6,7-dichlororiboflavin on germination. At a concentration of 20 μg/ml germination was completely inhibited. Mixtures of riboflavin and dichlororiboflavin gave results indicating that the latter compound was acting in a competitive manner

with riboflavin. The addition of 20 μg/ml dichlororiboflavin to a conidial suspension on 2% Difco Bacto agar completely suppressed germination.

(c) *Effect of Additions of Metabolites and Inorganic Nutrients on Germination and Germ-tube Elongation*

The effects of a number of compounds on germination and germ-tube elongation were tested on agar and in liquid suspension. Whenever possible, tests were made using the liquid suspension technique, in order that chemically defined conditions

TABLE 6

EFFECT OF SUGARS ON GERMINATION AND GERM-TUBE LENGTH IN LIQUID SUSPENSION

Compound	Germination (%)	Mean Germ-tube Length (μ)	Compound	Germination (%)	Mean Germ-tube Length (μ)
No sugar (control)	70	20·6	D-Melezitose	16	19·2
L-Arabinose	89	23·2	D-Raffinose	83	30·8
D-Lyxose	45	18·8	Turanose	66	18·8
D-Xylose	58	18·8	*gluco*Heptose	86	19·2
L-Rhamnose	24	20·4	Adonitol	73	26·0
L-Fucose	61	20·0	Dulcitol	60	18·6
D-Fructose	87	30·4	Erythritol	90	28·4
D-Glucose	90	19·6	Mannitol	90	26·0
D-Galactose	71	23·6	Sorbitol	91	26·0
D-Mannose	10	13·4	Inositol	53	18·0
L-Sorbose	69	23·6	2-Deoxyribose	28	22·0
D-Cellobiose	37	14·8	Gluconic acid	7	22·4
D-Lactose	74	20·8	D-Glucosamine	24	16·8
D-Melibiose	39	15·2	*N*-Acetyl-D-glucosamine	50	18·0
D-Sucrose	82	29·6			
D-Trehalose	75	21·6			
D-Maltose	83	25·2			
D-Gentiobiose	16	17·2			
L.S.D. (1%)	8·9	4·5			

might be used, but in some cases (most purines and pyrimidines, their analogues, and the amino acid analogues) the amounts of substances available precluded the use of this method and the basic agar technique alone was used.

Table 5 shows the effect of various vitamin additions on germination and germ-tube length. Both the standard agar and liquid suspension techniques were used, and in the latter additions were made to the basal medium described in Section II(c) above. On agar, the final concentration of all compounds was 25 μg/ml, and in liquid suspension, 4 μg/ml.

TABLE 7

EFFECT OF AMINO ACIDS AND THEIR ANALOGUES ON GERMINATION AND GERM-TUBE LENGTH

Compound	Agar Medium		Liquid Suspension	
	Germination (%)	Mean Germ-tube Length (μ)	Germination (%)	Mean Germ-tube Length (μ)
No additions (control)	67·2	18·4	82	19·2
Arginine	54·2	16·4	83	23·6
Alanine	70·8	23·2	74	20·0
Asparagine	73·4	28·8	82	24·6
Aspartic acid	77·5	29·6	84	22·8
β-Alanine	59·2	20·4	88	26·4
Cysteine	67·0	22·0	84	16·8
Citrulline	62·7	19·2	80	22·8
Glutamine	67·0	23·2	78	23·6
Glutamic acid	63·0	19·2	80	20·8
Glycine	55·6	20·0	78	20·4
Glutathione (reduced)	—	—	2	<1
Histidine	72·0	28·0	82	27·6
Hydroxyproline	63·0	17·6	75	16·8
α-Aminobutyric acid	74·4	28·0	82	24·4
γ-Aminobutyric acid	62·7	22·4	—	—
Isoleucine	78·1	28·0	78	27·2
Leucine	68·0	21·6	76	20·0
Lysine	74·3	31·2	72	26·0
Methionine	73·0	26·4	80	29·2
Ornithine	54·3	23·2	78	23·2
Proline	55·5	20·8	72	23·2
Phenylalanine	70·0	22·2	78	20·4
Serine	62·7	24·0	84	20·0
Threonine	76·0	32·8	88	24·4
Tyrosine	61·5	20·0	70	17·6
Tryptophan	74·4	29·6	85	29·4
Valine	70·0	20·0	69	24·4
Hydrolysed casein	85·2	29·2	80	27·2
Canavanine	44·2	12·6	23	17·2
Cysteic acid	61·2	19·2	61	20·0
Ethionine	68·6	28·8	—	—
β-Furylalanine	65·0	22·4	—	—
Homoseine	67·0	19·2	65	18·8
Homocysteine	65·0	22·4	—	—
α-Aminoisobutyric acid	62·7	22·4	—	—
Methionine sulphoxide	62·7	20·8	—	—
Norleucine	53·7	18·4	48	20·0
Norvaline	51·4	21·4	45	15·2
L.S.D. (1%)	8·2	4·7	8·1	15·2

The addition of vitamins produced no significant stimulatory effect either on germination or germ-tube length under either condition of incubation. However,

TABLE 8

EFFECT OF PURINES, PYRIMIDINES, AND THEIR ANALOGUES ON GERMINATION AND GERM-TUBE LENGTH ON AGAR

Compound	Mean Germination (%)	Mean Germ-tube Length (μ)	Compound	Mean Germination (%)	Mean Germ-tube Length (μ)
None (control)	85·5	21·0	5-Methylorotic acid	89·0	17·2
Guanine	86·5	30·4	6-Mercaptopurine	86·0	17·6
Guanosine	86·5	28·4	Theophylline	85·5	15·6
Guanylic acid	67·5	22·8	Theobromine	79·5	11·6
Isopropylidine guanosine	66·5	20·4	Caffeine	34·5	8·0
Isoguanine sulphate	78·5	26·8	2,6-Diaminopurine	89·0	18·8
6-Azaguanine	71·5	29·6	8-Aza-2,6-diaminopurine	79·5	16·4
Thymine	84·5	22·4	Uracil	85·0	31·2
Dihydrothymine	69·0	30·0	Uracil-5-carboxylic acid	61·5	16·2
Thymidine	72·0	30·4	5-Aminouracil	92·5	25·2
Dithiothymine	70·0	16·0	Diazauracil	90·5	27·2
6-Azathymine	83·0	31·2	Thiouracil	98·5	30·0
5-Methylcytosine	84·5	25·4	Sulphaminouracil	87·5	21·6
Deoxycytidine	81·5	19·6	Uric acid	87·0	23·6
Cytidylic acid	88·0	27·2	Uracil-4-acetic acid	77·0	12·8
Cytidine	77·0	16·8	Uridine	99·0	15·6
2-Thiocytosine	88·0	25·0	Uramil	82·0	30·0
Isocytosine	74·5	19·6	Dithiouracil	75·0	26·4
Cytosine	83·0	25·4	5-Nitrouracil	3·0	0
Xanthosine	83·0	20·0	Propylthiouracil	81·0	26·8
8-Azahypoxanthine	68·0	14·8	6-Azauracil	85·0	26·0
Hypoxanthine	82·0	30·0	6-Methyluracil	95·0	32·0
Xanthine	86·5	20·8	Uridylic acid	74·0	20·8
8-Azaxanthine	88·0	18·4	Oxaluric acid	85·0	28·4
8-Chloroxanthine	57·0	14·8	Inosine	76·0	20·8
Adenine	79·0	23·6			
Adenosine	82·5	20·4			
Adenylic acid	83·5	20·8			
Adenosine diphosphate	78·0	26·0			
Adenosine triphosphate	78·0	20·0			
8-Aza-adenine	70·5	18·4			
Desoxyadenosine	79·0	21·2			
Isopropylidine adenosine	78·0	14·8			
L.S.D. (1%)				9·0	7·1

addition of vitamins to conidia tested by the standard agar technique produced a general depression of germ-tube growth, which did not occur under the chemically defined conditions of the liquid suspension test.

Because of the possibility of small amounts of soluble carbohydrate being present in agar, tests on the effect of carbohydrates on germination and germ-tube elongation were made in liquid suspension only, the results being shown in Table 6. Incubation was for 3 hr at 15°C, the sorbitol in the basal medium (see Section II(*c*)) being replaced by the sugars indicated in the table. All compounds were tested at a final concentration of 200 μg/ml.

It may be seen (Table 6) that many of the sugars tested produced significant stimulations, or inhibitors, of both germination and germ-tube length.

The effects on germination and germ-tube length of additions of various amino acids and their analogues are shown in Table 7. Compounds were tested by both the basic agar and liquid suspension techniques. In both cases, the final concentration

TABLE 9

EFFECT OF PURINE AND PYRIMIDINE ADDITIONS ON GERMINATION AND GERM-TUBE LENGTH IN LIQUID SUSPENSION

Compound	Germination (%)	Mean Germ-tube Length (μ)
None (control)	79	26·4
Guanine	77	40·8
Thymine	75	37·2
Cytidine	87	37·8
Hypoxanthine	90	50·8
Xanthine	76	26·4
Adenine	89	37·2
Uracil	94	55·6
Inosine	46	37·4

of amino acid was 100 μg/ml and incubation was for 3 hr at 15°C. In the liquid suspension tests, 200 μg/ml of potassium nitrate was present in the medium. No requirement for a specific amino acid for germination was recorded, but the results indicate the stimulation of germination in the presence of an available nitrogen source. In both tests, germ-tube elongation was significantly stimulated by asparagine, glutamine, histidine, α-aminobutyric acid, isoleucine, lysine, methionine, ornithine, threonine, and tryptophan. In the agar tests, canavanine, norleucine, and norvaline were the only analogues to exhibit significant inhibition of germination, whereas all analogues tested were somewhat inhibitory to germination in the liquid suspension tests.

The effects of purines, pyrimidines, and their analogues, when tested by the basic agar technique, are shown in Table 8. All compounds were present at a final concentration of 100 μg/ml and incubation was for 4 hr at 15°C.

No very marked stimulation of germination was observed on agar, but guanylic acid, isopropylidine guanosine, 6-azaguanine, dihydrothymine, thymidine, dithiothymine, 8-azahypoxanthine, 8-chloroxanthine, 8-aza-adenine, caffeine, and

5-nitrouracil showed significant inhibitory effects. Germ-tube elongation was significantly stimulated by guanine, dihydrothymine, thymidine, 6-azathymine, hypoxanthine, thiouracil, uracil, uramil, and 6-methyluracil and was significantly inhibited by 8-azahypoxanthine, 8-chloroxanthine, isopropylidine adenosine, theobromine, caffeine, uracil-4-acetic acid, and 5-nitrouracil.

A more limited series of compounds was tested by the liquid suspension technique, the results being shown in Table 9. In this case conidia were incubated for 3 hr at 15°C in the basal medium (Section II(*c*)) and compounds were added to give a final concentration of 100 μg/ml.

TABLE 10

EFFECT OF ADDITIONS OF RIBOFLAVIN, SORBITOL, ASPARAGINE, AND INORGANIC SALTS ON GERMINATION ON AGAR

0 indicates absence of constituent and + indicates presence of constituent in medium

Medium Constituents	Concn. (μg/ml)	Medium No.									
		1	2	3	4	5	6	7	8	9	10
Sorbitol	500	0	+	0	+	0	0	+	+	+	+
Riboflavin	20	0	0	+	+	0	+	+	+	+	+
Asparagine	400	0	0	0	0	+	+	+	+	+	+
$MgSO_4.7H_2O$	200	0	0	0	0	0	0	0	+	0	+
Na_2HPO_4	100	0	0	0	0	0	0	0	0	+	+
Substrate		Germination (%) after 4 hr Incubation at 15°C									
2% "washed" Difco Bacto agar		11	20	75	85	32	87	80	84	86	80
2% Difco Bacto agar		79	74	83	80	83	79	80	85	77	75

In the liquid suspension tests, hypoxanthine and uracil showed marked stimulations of both germination and germ-tube length, the latter also being stimulated by guanine and adenine. No inhibitory effects were observed.

The results presented in Tables 6 and 7 suggested that the presence of available sources of carbon and nitrogen enhanced germination and germ-tube growth. Tables 10 and 11 present more definitive evidence to substantiate this suggestion, together with results on the effects of additions of various inorganic salts. Table 10 shows the effects of carbon and nitrogen sources and some inorganic salts when these are added to conidia on solid substrata. 0·05 ml of conidial suspension was placed on either 2% Difco Bacto agar, or 2% washed agar and 0·05 ml of solution, containing the compounds indicated in the table, added. Incubation was for 4 hr at 15°C. Table 11 shows the results from similar experiments conducted in liquid suspensions, these having the compositions indicated in the table. Incubation was at 15°C and samples were withdrawn at intervals for assessment of germination. Germ-tube length was assessed after 4 hr incubation.

The results presented in Table 10 indicate that 2% Difco Bacto agar contains all the necessary nutrients to allow a high degree of germination. With washed agar as the substratum, however, the degree of germination is markedly stimulated by riboflavin and the presence of further nutrients has only a small additional stimulatory effect (cf. medium 3 with media 4, 6, 7, 8, 9, and 10).

When chemically defined media are used, both the degree of germination after 3 hr incubation and the rate of germination over a 5-hr period are affected by the presence of a number of constituents of the medium. The results presented in Table 11 indicate that germination is stimulated in the presence of riboflavin,

TABLE 11

EFFECT OF RIBOFLAVIN, SORBITOL, AND INORGANIC SALTS ON GERMINATION AND GERM-TUBE LENGTH IN LIQUID SUSPENSION

0 indicates absence of constituent and + indicates presence of constituent in medium

Medium Constituents	Concn. (μg/ml)	Medium No.												
		1	2	3	4	5	6	7	8	9	10	11	12	13
Sorbitol	1000	0	0	+	+	+	+	+	+	+	+	0	+	+
Riboflavin	20	0	+	0	+	+	+	+	+	+	+	+	+	0
$MgSO_4.7H_2O$	200	0	0	0	0	+	0	+	+	0	+	+	+	+
$CaCl_2$	200	0	0	0	0	0	+	+	0	+	+	+	+	+
KNO_3	200	0	0	0	0	0	0	0	+	+	+	+	+	+
Na_2HPO_4	500	0	0	0	0	0	0	0	0	0	0	0	+	+
Incubation Time (hr)		Germination (%) after Incubation at 15°C												
0		0	0	0	0	0	0	0	0	0	0	0	0	0
2		0	0	0	0	2	1	0	2	0	10	32	75	0
3		2	2	1	5	19	23	26	30	34	68	57	90	12
4		7	12	3	17	48	57	58	65	62	81	68	91	18
5		13	21	5	22	76	69	80	86	74	87	75	90	27
		Germ-tube Length (μ) after 4 hr Incubation at 15°C												
		—	—	—	15	18	26	24	18	20	24	26	30	—

sorbitol, magnesium sulphate, calcium chloride, potassium nitrate, and sodium phosphate, and that the omission of any one of these components reduces the rate of germination. The germ-tube length at 4 hr is markedly reduced when calcium chloride is omitted from the medium. Replacement of calcium chloride by an equivalent amount of strontium chloride in medium 12 produced an almost complete inhibition of germination. Magnesium sulphate could be replaced by magnesium chloride, or magnesium nitrate, without any detectable effect, but replacement with potassium sulphate reduced both the degree of germination and the germ-tube

length, e.g. in the presence of magnesium sulphate the degree of germination and germ-tube length after 5 hr incubation in medium 12 were 90% and 39·6 μ respectively, whereas in the presence of potassium sulphate these values fell to 43% and 22·8 μ.

TABLE 12

EFFECT OF VARIOUS PHOSPHATE SOURCES ON GERMINATION AND GERM-TUBE LENGTH

Medium 12 (see Table 11) used as basal medium

Phosphate Source (12·5 μg P/ml)	Germination (%) after Incubation for 3 hr	Mean Germ-tube Length (μ) after Incubation for 4 hr
Disodiumhydrogen orthophosphate	90	26·0
Sodium pyrophosphate	72	18·8
Sodium hexametaphosphate	44	16·8

With medium 12 as the basal medium, further experiments were carried out on the effects of various phosphate and nitrogen sources on germination and germ-tube length, the results being shown in Tables 12 and 13 respectively. Ortho-

TABLE 13

EFFECT OF VARIOUS NITROGEN SOURCES ON GERMINATION AND GERM-TUBE LENGTH

Medium 12 (see Table 11) used as basal medium

Nitrogen Source (30 μg N/ml)	Germination (%) after Incubation for 3 hr	Mean Germ-tube Length (μ) after Incubation for 4 hr
Potassium nitrate	88	24·0
Potassium nitrite	2	—
Ammonium sulphate	66	14·4
Asparagine	92	23·6

phosphate gave higher germination than either pyro- or hexametaphosphate, while nitrate and asparagine gave a greater germination than ammonia or nitrite.

The addition to medium 12 (Table 11) of 5 μg/ml of iron, copper, manganese, molybdenum, cobalt, zinc, and boron produced no detectable effects on the rate of germination measured during incubation at 15°C. However, the addition of 200 μg/ml of ethylenediaminetetraacetic acid to medium 12 completely inhibited germination.

The addition to medium 12 (Table 11) of acetic, fumaric, succinic, malic, citric, lactic, and tartaric acids as the sodium salts at a concentration of 200 μg/ml produced no detectable effects on the rate of germination, but propionic acid was inhibitory (germination of control after 2 hr incubation at 15°C was 90%; with 200 μg/ml propionic acid, 34%).

The addition of potassium carbonate or potassium bicarbonate at a concentration of 200 μg/ml to medium 12 (Table 11) produced no significant effects.

(*d*) *Effect of Tobacco Exudates and Extracts on Germination on Agar*

Exudates from leaves of *N. tabacum* cv. Virginia Gold were prepared according to Cruickshank (1961). Guttation droplets were collected from the leaves of similar plants. Leaf extracts were made by cutting from the leaf lamina 10 disks of 15 mm diameter and blending with 5 ml of water in a microhomogenizer. The solutions were clarified by centrifugation at 2000 *g* for 20 min and the supernatants tested for their effects on germination by the basic agar technique. The results of this study are shown in Table 14.

TABLE 14
EFFECT ON GERMINATION OF EXTRACTS AND EXUDATES OF TOBACCO LEAVES

Nature of Extract	No. of Replicates	Mean Germination (%)
Guttation fluid	10	80·0±8·0
Leaf exudates	40	79·5±7·8
Leaf extracts	100	80·9±6·1
Water control	90	88·0±5·4

Thus exudates and extracts of tobacco leaves were shown to have no significant effect on the degree of germination, when tested by the basic agar method. Similarly, no significant effects were recorded when germ-tube length was measured after 4 hr incubation at 15°C (mean germ-tube length of water controls 43·1 μ; mean length in presence of leaf extracts 40·0 μ). When the degree of germination was assessed at intervals during the incubation period, no effect on the rate of germination was observed in the presence of leaf exudates and extracts.

(*e*) *Effect of Temperature of Incubation on Germination*

The effects of various temperatures on the rate of germination of conidia on 2% Difco Bacto agar and in liquid suspension (in medium 12, Table 11) are shown in Figures 2 and 3. In both, a temperature optimum in the range 15–20°C was observed, the rate of germination being lower at temperatures outside this range. Some germination occurred after 4 hr at 0 and 30°C (2 and 5% respectively, when tested on agar), but none at 35°C in either agar or liquid suspension tests.

(f) Effect of pH of Medium on Germination

Germination was found to be affected by changes in the pH of the medium in which conidia were suspended (Fig. 4).

In the agar tests, conidia were suspended in 0·01M phosphate–citrate buffer (McIlvaine) of the appropriate pH before being placed on 2% Difco Bacto agar.

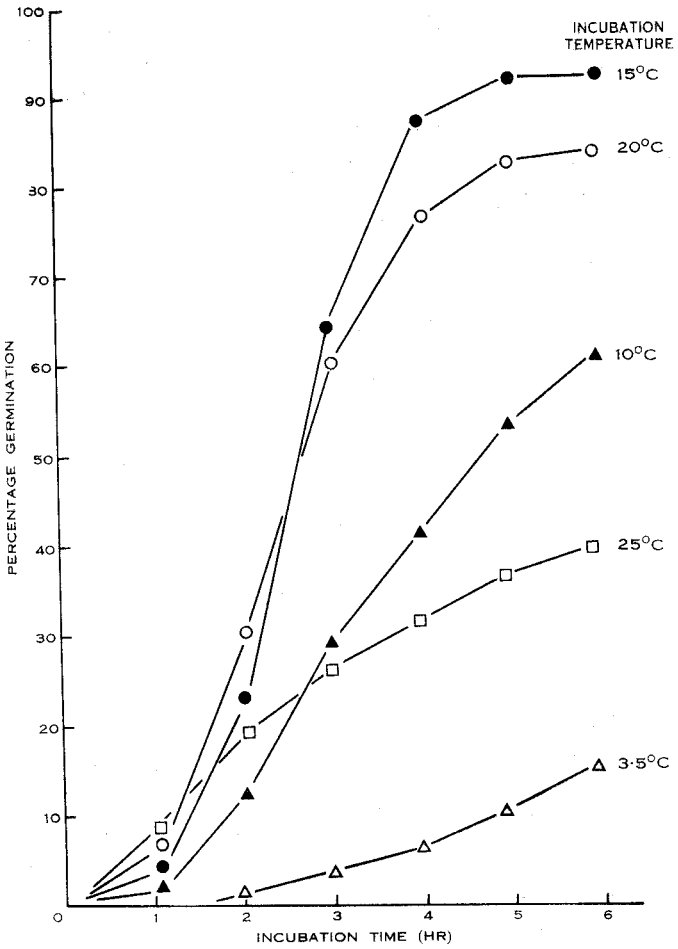

Fig. 2.—Effect of temperature on rate of germination on agar.

With glass as the substratum, the pH of the suspending medium (20 μg/ml riboflavin solution) was adjusted with either HCl or KOH. When the liquid suspension technique was used, the basal medium was similarly adjusted with HCl or KOH, before suspension of the conidia. All pH values were checked at the termination of the experiment, and in all cases the changes occurring did not vary more than ±0·2 pH units from the initial values.

On agar, a broad optimum was found in the range pH 5·5–8·0. In liquid suspension, the optimum was in the range pH 6·5–8·0, whereas on glass in the presence of riboflavin the optimum range found was pH 6·5–7·5.

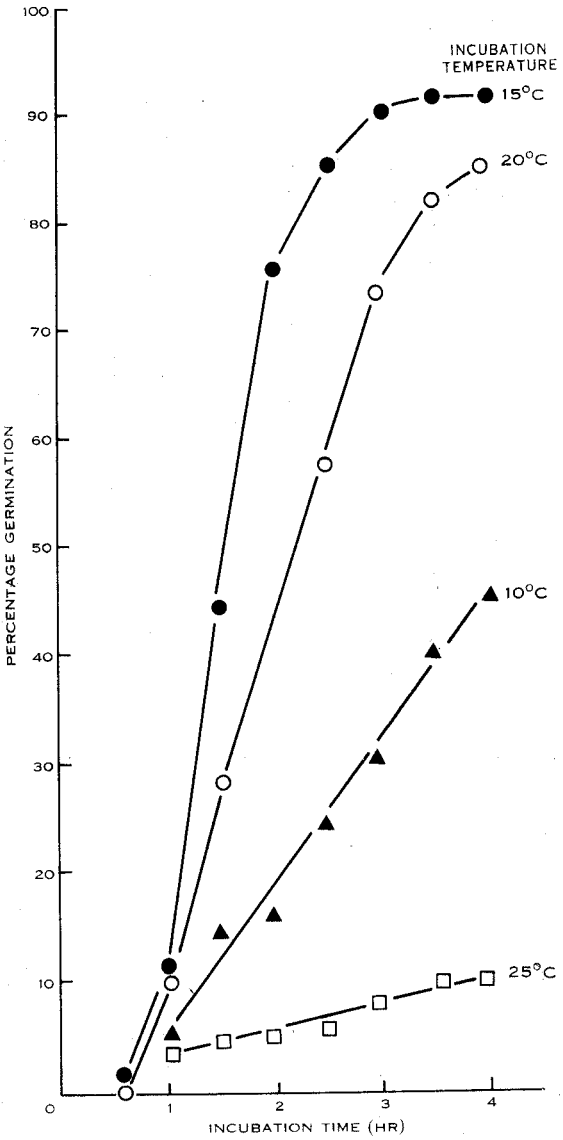

Fig. 3.—Effect of temperature on rate of germination in liquid suspension.

(*g*) *Effect of Age of Conidia on Germination*

Infected tobacco plants were induced to sporulate under the conditions described in Section II(*a*), and the conidia were collected at intervals after induction.

During the experiment, the plants were kept at 20°C and a relative humidity approaching saturation. Although it might be expected that sporulation would continue throughout the period of the experiment, the results indicate that conidial germinability decreases after 24 hr. Mean germination was assessed after incubation on 2% Difco Bacto agar for 4 hr at 15°C, and was found to be 85·0, 61·7, and 47·8% for the periods 17, 41, and 65 hr after induction of sporulation.

During the period 17–29 hr after the induction of sporulation, conidia were collected at various times of the day (8 a.m., 12 a.m., 4 p.m., and 8 p.m.). No significant differences in germination were observed in these different collections, supporting the data of Armstrong and Sumner (1935) and not in agreement with that of Clayton and Gaines (1945).

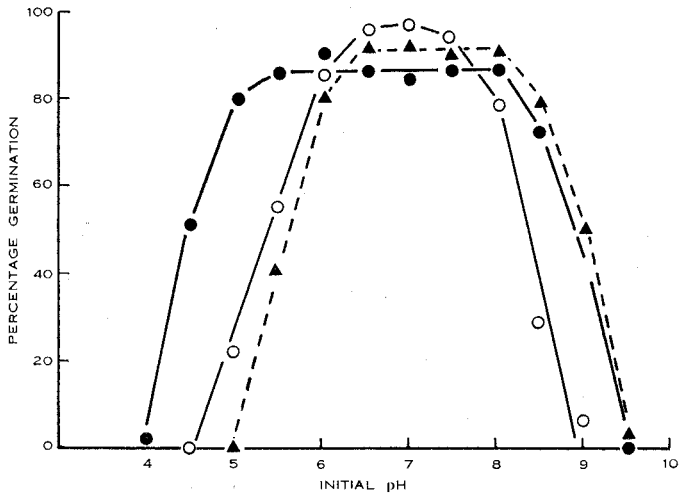

Fig. 4.—Effect of pH of medium on germination. Incubation was for 4 hr at 15°C. ●On 2% agar. ○On glass with riboflavin. ▲In liquid suspension.

(*h*) *Effect of Resporulation of Leaves on Germination*

Infected tobacco plants were induced to sporulate under the conditions described in Section II(*a*). 17 hr after the initiation of sporulation, the conidia were removed as completely as possible from the leaves by washing with a jet of water. The plants were again induced to sporulate and conidia were again washed from the same leaves 41 hr after the initial induction. A third crop of conidia was produced similarly and removed 65 hr after the initial induction time. The mean germination of conidia produced at these three times was assessed after incubation on 2% Difco Bacto agar for 4 hr at 15°C and found to be 84·4, 32·1, and 3·1% respectively. These results indicate that the ability of successive crops of conidia to germinate decreases markedly.

(i) Effect of Storage of Conidia in Water on Germination

A conidial suspension was washed twice by centrifugation in distilled water, and the conidial sediment was allowed to stand undisturbed at 15°C under the supernatant of the second centrifugation. At intervals, the conidia were resuspended in distilled water, and the germination assessed after incubation for 4 hr at 15°C on 2% Difco Bacto agar.

No germination was observed at any time in the conidial sediment, presumably because riboflavin was limiting. The results, shown in Table 15, indicate that storage, under these conditions, for 6·5 hr does not affect the germination, but that this is reduced by further storage.

TABLE 15

EFFECT OF STORAGE OF CONIDIA IN WATER ON GERMINATION

Storage time (hr)	0	1	2	4	6·5	10·5	18
Mean germination (%)	87·9	82·6	91·3	82·3	87·1	54·5	0·6

(j) Effect of Visible and Ultraviolet Irradiation on Germination

Conidia were incubated, using the basic agar method, for 4 hr at 15°C in darkness, and under two different intensities of fluorescent lighting. The mean germinations obtained—84·4% (darkness), 84·8% (dim light, 2 f.c.), and 78·6% (bright light, 460 f.c.)—indicate that visible light did not affect germination.

A suspension of 10^5 conidia per ml was placed in a quartz dish and irradiated with a Hanovia ultraviolet lamp, while being rocked continuously. At intervals, samples of the suspension were withdrawn and the degree of germination assessed, using the basic agar method, after incubation for 6 hr at 15°C. The ultraviolet dosage, at all wavelengths below 4000 Å, was calculated from data supplied by the manufacturers of the lamp, and the percentage of surviving conidia was calculated after correction for the degree of germination found in unirradiated conidia (92·7%). The results, shown in Figure 5, are characteristic of a multi-hit target curve, and it was demonstrated graphically that there is a mean number of 17·5 targets per conidium.

(k) Physical Appearance of Germinating Conidia

Few observations have been recorded on the physical appearance of germinating conidia of *Peronospora tabacina*. Angell and Hill (1932) and Adam (1933) noted that the germ tubes usually arise from the sides of the conidia, but the former authors state that occasionally germ tubes are produced at the end by which the conidia were previously attached to the conidiophores. During the course of the present study, it has been observed that germ-tube formation occurs most frequently at the sides of the conidia, but a germ tube may also be produced at any point on the conidial surface, and rarely, two germ tubes are produced by a single conidium. Plate 1, Figures 1–4, and Plate 2, Figure 1, illustrate the sequence of events occurring during

germination on agar. Measurement of conidia has indicated that there is no swelling prior to germination. The first recognizable indication of germination is the formation of a small papilla on the conidial wall (Plate 1, Fig. 2). This papilla rapidly extends to form a thin-walled germ tube (Plate 1, Fig. 3) and, as elongation proceeds, the protoplasm in the conidium flows into the germ tube (Plate 1, Fig. 4) until the

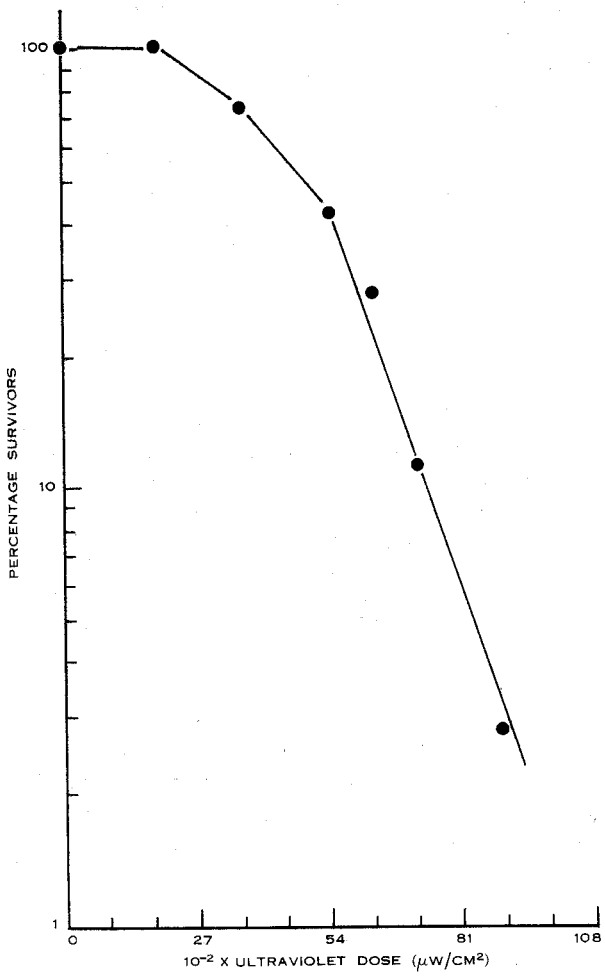

Fig. 5.—Effect of ultraviolet dosage on viability of conidia.

conidium is finally empty of its contents (Plate 2, Fig. 1). Branching of the germ tube occurs at about this time when conidia are germinated under optimal conditions. In the liquid suspension experiments, germ tubes were produced that were extensively branched, and the final total length of mycelium obtained was in the range 400–450 μ. Henderson (1937) has observed a similar sequence of events in conidia germinating on tobacco leaves.

When germination occurred on glass, or in liquid suspension in the absence of calcium ions, the frequent production of appressoria-like structures was observed. The occasional appearance of such structures has been noted by previous authors (Angell and Hill 1932; Henderson 1937). Germination on glass, in the presence of limiting amounts of riboflavin, gave rise to the production of tortuous and spiralling germ tubes (Plate 2, Fig. 2). Wolf *et al.* (1934) observed a similar production of abnormal germ tubes when conidia were germinated in water on glass slides, and it may be inferred that this phenomenon was connected with the presence of an inadequate amount of riboflavin.

During the germination of many fungi, the germ tubes exhibit a negative tropism toward each other (Stadler 1952), but this phenomenon has never been observed during the germination of *P. tabacina* conidia on solid substrata (Plate 2, Fig. 3).

IV. Discussion

(*a*) *Effect of Chemical Factors on Germination and Germ-tube Growth*

The most frequent comment in the literature regarding the germination of spores of *P. tabacina* has been on the extreme variability encountered (Angell and Hill 1932; Wolf *et al.* 1934; Armstrong and Sumner 1935; Clayton and Gaines 1945). This variability was overcome by the use of washed conidial suspensions germinated in the presence of complex nutrient materials (Cruickshank 1961).

The present results (Table 4 and Figure 1) suggest that riboflavin is required for conidial germination. Supporting evidence for this statement is that the analogue isoriboflavin can also satisfy this requirement but is less effective than riboflavin, whereas the analogue 6,7-dichlororiboflavin was shown to act as a competitive antagonist to riboflavin. As washed conidia were used in these experiments it must be admitted that the method is open to the objection that essential materials may be leached from the spore during the washing process. However, the washing process is obligatory in order to remove the water-soluble inhibitor present in unwashed conidia. Thus it is impossible to demonstrate in an unequivocal manner that conidia have a natural riboflavin requirement, although the author considers that this is most probable.

Previous observations that germination on glass slides is very variable may perhaps be explained on the basis of inadequate riboflavin and the presence of germination inhibitor, where unwashed spores were used, both of which would reduce the degree of germination attainable under optimum conditions. Where washed spores were used, it may be inferred that the absence of an adequate amount of riboflavin was the controlling factor. However, in a few instances high levels of germination (up to 80%) have been recorded under the latter condition. This fact makes it necessary to postulate that the conidial content of riboflavin may vary from batch to batch, this presumably being conditioned by host–environment factors. The observations of Clayton and Gaines (1945) on the effects of plant age and environment on germination lend support to this thesis.

Few natural vitamin requirements for germination have been reported, the only known requirement for riboflavin being in the case of *Colletotrichum gloeosporioides* (Cooper 1939).

In addition to riboflavin, stimulation of the rate of germination under chemically defined conditions has been shown for various other substances (Table 11). These effects were obscured when agar was used as the substratum for germination. In some cases (nitrogen, carbon, and phosphate sources present) the observed stimulations might be interpreted as indicating a partial dependence on external nutrients. However, such interpretation is open to the same objections as in the case of the apparent riboflavin requirement. The responses to calcium and magnesium ions are not necessarily nutritional, as conidia are so sensitive to heavy metal ions that the divalent metals may act primarily as anti-inhibition agents by depressing the accumulation of toxic ions (Marsh 1945). However, the fact that neither calcium nor magnesium can be replaced by additional amounts of each other, nor by other divalent ions, and the observed effects of both on germ-tube growth perhaps indicate something more than a non-specific action of these ions.

Tests in liquid suspension and on agar (Table 5) have indicated that the addition of vitamins, other than riboflavin, produced no significant effects either on germination or on germ-tube elongation. Significant effects on germination were recorded with various sugars (Table 6). Fructose, sucrose, maltose, raffinose, erythritol, mannitol, and sorbitol showed stimulation of germination and germ-tube elongation, while mannose, cellobiose, and melibiose significantly inhibited both processes. In addition, germination alone was shown to be sensitive to stimulation and inhibition by a number of other sugars and their derivatives. While it may be inferred that conidia are permeable to compounds showing stimulatory effects, no rationalization can be made at present in the case of those compounds showing inhibition.

No requirements for specific amino acids for germination were recorded (Table 7), but these results, together with those presented in Tables 10 and 11 indicate the stimulation of germination in the presence of an available nitrogen source. Germ-tube elongation, however, was significantly stimulated by the presence of a number of amino acids. No absolute requirement was found, and the results may perhaps be interpreted as evidence of limiting rates of synthesis of these compounds by the conidia. Similarly, in the agar tests, no significant stimulations of germination were observed on the addition of various natural purines and pyrimidines (Table 8), although significant stimulations were produced by hypoxanthine and uracil in liquid suspension (Table 9). These compounds, together with guanine, dihydrothymine, thymidine, 6-azathymine, thiouracil, uramil, and 6-methyluracil significantly increased germ-tube elongation. If the assumption is made that agar contains trace amounts of available purines and pyrimidines, then the differences in germination responses found on agar and in liquid suspension may be readily interpreted. A number of analogues of natural purines and pyrimidines were shown to be inhibitory to both germination and germ-tube elongation. While the possibility of selective permeability of conidia to the compounds tested makes interpretation of the results difficult, the effects demonstrated in the case of uracil and its derivatives (Table 8) suggest the possibility of limiting synthesis of this compound in germ-tube elongation.

It was surprising to find (Table 13) that ammonium nitrogen gave a lower degree of both germination and germ-tube elongation than either nitrate or amino acid nitrogen. It is possible that selective permeability to the ions occurred under the conditions of the test.

While Cruickshank (1961) reports the stimulation of germination of conidia by exudates of tobacco leaves, no such effect was observed during the present investigation (Table 14). However, the data presented in Table 3 indicate that germination was lower on 2% Difco prune agar, as used by Cruickshank (1961), than on 2% Difco Bacto agar. Thus, the possibility exists that some component is limiting in prune agar that is present in adequate quantities in Bacto agar. If this component is present in tobacco exudates, then a stimulating effect might be expected on prune agar, but not on Bacto agar. The identity of the component showing this stimulatory effect is unknown, but it could well be one or more of those substances listed in Table 11.

TABLE 16
EFFECT OF TEMPERATURE ON RATE OF GERMINATION

Temperature (°C)	Approximate Rate of Germination (%/hr):	
	On Agar	In Liquid Suspension
3·5	4	—
10	12	13
15	41	64
10	30	29
25	4	3

(b) Effect of Physical Factors on Germination and Germ-tube Growth

The response of germination of fungal spores to temperature is known to be affected by a variety of factors, such as the time of observation (Felton and Walker 1946), the pH of the medium (Tilford 1936), and the nutrient supply (Yarwood *et al.* 1954). Thus the appearance in the literature of differing optimal temperature ranges for the germination of *P. tabacina* conidia is hardly surprising. In the present study, an optimum in the range 15–20°C was observed, both on agar and in liquid suspension. While this is so when the amount of germination recorded after a fixed time of incubation is considered, the data presented in Figures 2 and 3 are capable of further analysis. If the maximum rate of germination is the criterion, then the optimum is more clearly at about 15°C (Table 16) both for germination on agar and in liquid suspension. Thus the rate of germination is more sensitive to non-optimal temperatures than is the final germination.

Similarly the early processes of germination show a temperature response pattern differing from that of final germination. When the data from Figure 2 are used to indicate the time taken before 3% germination has occurred, this arbitrary figure being defined as the "latent period" after incubation at various temperatures,

it may be seen (Fig. 6) that the early processes of germination are most favoured by the highest temperature employed (25°C) and that lower temperatures give a correspondingly increased latent period before this degree of germination is attained. It may be suggested, therefore, that the early metabolic reactions of conidial germination have a different response to temperature than later processes. A similar differential effect of temperature on the early and late phases of germination has been shown by Cochrane (1945) for uredospore germination of *Phragmidium mucronatum*. Felton and Walker (1946) have reported an optimum temperature range of 10–16°C for *Peronospora parasitica*, but have noted that initiation of germination was most rapid between 8–12°C, germination being reduced at 20°C and none occurring at 28°C.

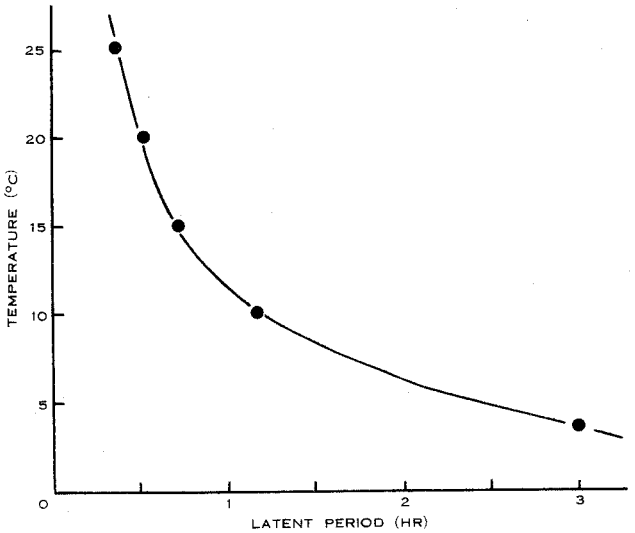

Fig. 6.—Effect of temperature on "latent period" of germination.

Similarly to temperature response, the form of the pH response curve of fungi is affected by various factors, including the previous history of the spore population (Allen 1955), the presence of nutrient materials (Butler 1956), and the type of buffer used (Sussman 1954). The differing response curves shown in Figure 4 may therefore be interpreted in terms of differing buffer and nutrient status. It will be noted that the optimum on glass was most restricted, where the nutrient status of the conidia was restricted, wider optima being observed where a more adequate nutrient supply was available. In all media, however, considerable germination occurred in the range pH 7–8, and in liquid suspension 50% germination was recorded at pH 9·0. This is in sharp contrast to the results of Wolf *et al.* (1934), who reported a broad optimum pH range of 3·6–7·0, and a much reduced degree of germination outside this range. It may be inferred, from the technique used, that these authors were employing conditions of suboptimal riboflavin status.

The present study has confirmed the observation of Wolf *et al.* (1934) that conidia will germinate in both light and darkness. The report by Angell and Hill

(1932) that conidia rapidly become inviable after exposure to sunlight may perhaps be interpreted in terms of their sensitivity to ultraviolet irradiation. If the assumption is made that conidial inactivation is the result of nuclear inactivation, it may be calculated from the ultraviolet dosage–response curve that there is a mean number of 17–18 nuclei per conidium. Following cytological examination, Mandryk (personal communication) has reported nuclear numbers per conidium of 8–22, with a mean number of 15.

There is no good reason to doubt that the effects of chemical and physical factors on germination *in vitro* might not obtain *in vivo* when germination occurs on the tobacco leaf, for the effects reported are intrinsic properties of *P. tabacina* conidia. However, ignorance of the precise physicochemical environment on the leaf surface precludes any extrapolation of the present results to the *in vivo* situation. Observations on the latter will be reported in a subsequent paper.

V. Acknowledgments

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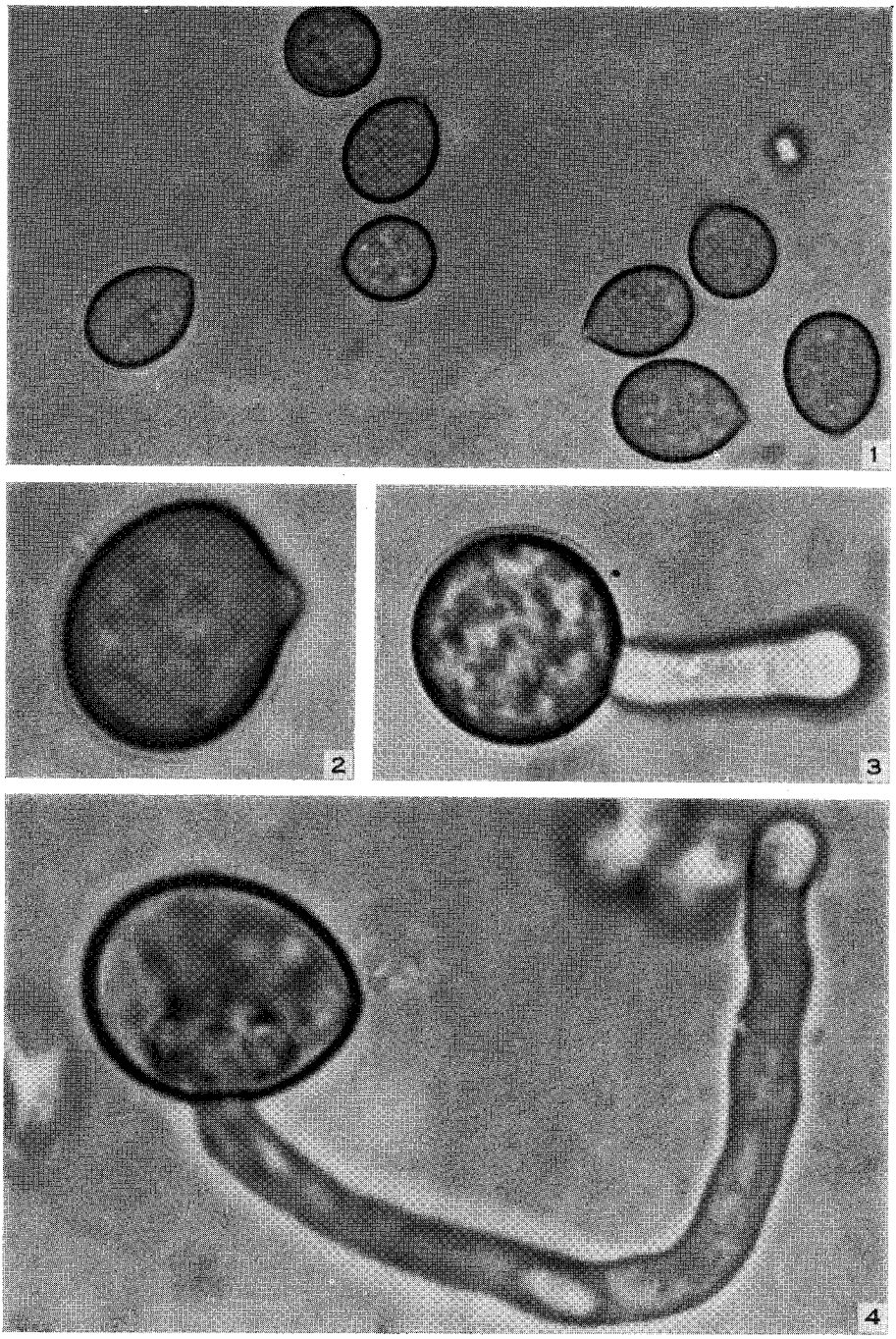
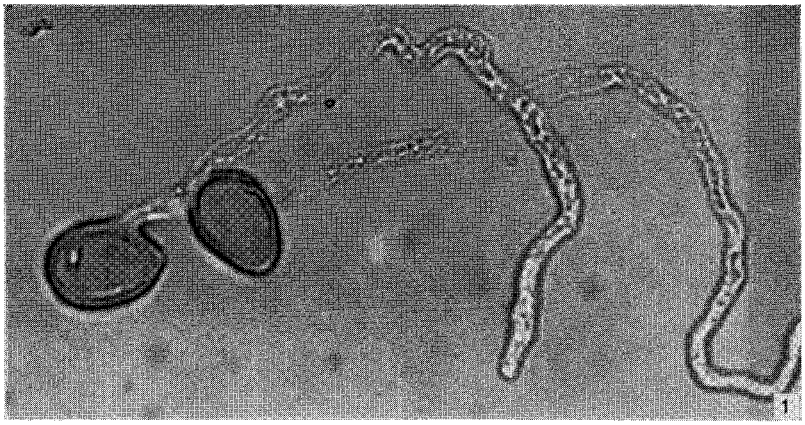
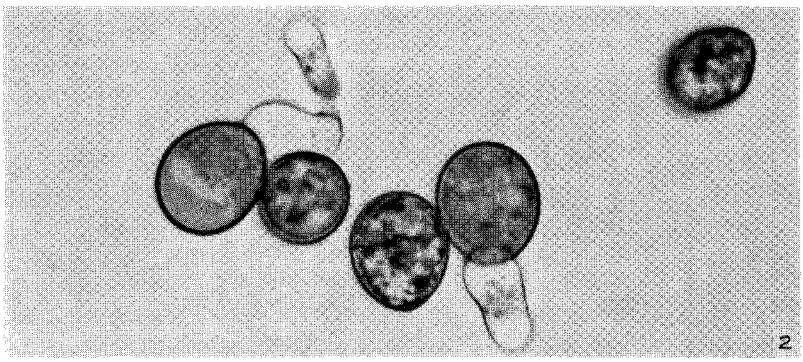
GERMINATION OF CONIDIA OF PERONOSPORA TABACINA. I



Figs. 1–4.—Physical appearance of germinating conidia: *1*, Ungerminated conidia. *2*, Appearance of germ-tube papilla. *3*, Germ-tube formation. *4*, Conidial contents passing into germ tube.

GERMINATION OF CONIDIA OF PERONOSPORA TABACINA. I





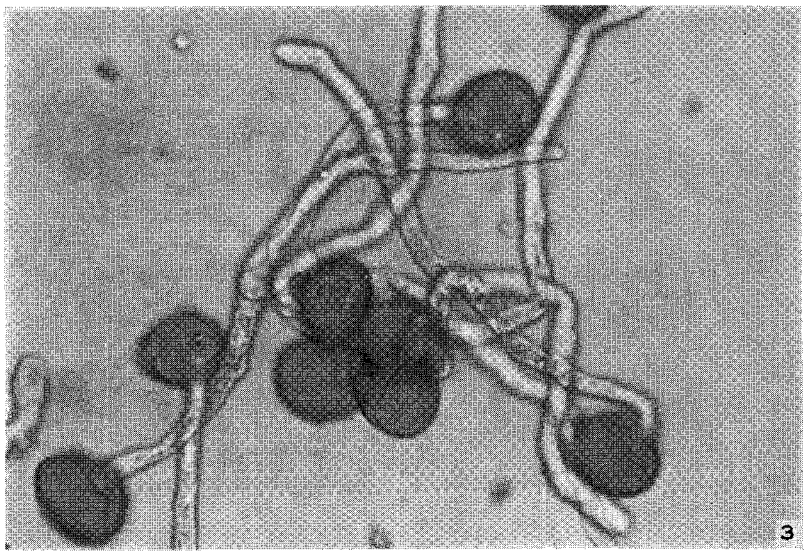


Fig. 1.—Conidial contents entirely in germ tube.
Fig. 2.—Spiralling of germ tube on glass with riboflavin limiting.
Fig. 3.—Seven hours after start of germination, showing lack of mutual inhibition of germ tubes.