Comparative Studies of the Mineral Nutrition of Three Species of *Phytophthora*

By P. G. FOTHERGILL

Department of Botany, University of Newcastle-upon-Tyne

AND J. H. CHILD

Department of Chemistry and Applied Chemistry, Royal College of Advanced Technology, Salford

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SUMMARY

Nutritional experiments were carried out dealing with: (1) the utilization of sulphur compounds; (2) the utilization of phosphorus compounds; (3) the action of different mineral salts and their interaction. The results showed that *Phytophthora erythroseptica* grew well at 28° and pH 6.6 incubated for 20 days; *P. parasitica* grew well at 28° and pH 6.6 for 17 days; and *P. infestans* at 20° and pH 4.5 for 16 days. In the adjusted controlled medium pH changes were generally within one pH unit. The best carbon and nitrogen sources are stated. The only satisfactory sulphur sources for *P. infestans* were sodium sulphate and sodium thiosulphate. These compounds were also among the best S sources for *P. erythroseptica* and *P. parasitica*, but a number of other compounds also were utilized equally well, e.g. sodium metabisulphite, sodium sulphite, α -cysteine, sodium sulphide, sodium dithionite and methionine. For all organisms the best phosphorus sources were sodium dihydrogen orthophosphate, sodium metaphosphate and lecithin. Rate of utilization of the phosphorus was an important factor in mycelial yield. Factorial experiments were carried out in which *P. erythroseptica* and *P. parasitica* were incubated at 28° for 17 days. Statistical analysis of the results showed that under the given conditions optimal growth measured as mg. dry wt. was obtained in liquid media containing glucose, 25 g./l.; DL-asparagine, 4.0 g./l.; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.001 g./l.; thiamine, 0.8 mg./l.; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 1 p.p.m.; H_2MoO_4 , $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.02 p.p.m.; with varying amounts of K_2HPO_4 , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ for the different species specified. There was a balance between all combinations of K_2HPO_4 , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ for *P. parasitica* but not for *P. erythroseptica*, but there were significant interactions between the salts taken two at a time for both these fungi. There was interaction for all three salts together for both species. *Phytophthora infestans* and *P. parasitica* are more exacting in their nutritional requirements than *P. erythroseptica*.

INTRODUCTION

Previous work at Newcastle-upon-Tyne (see Fothergill & Raine, 1954; Fothergill & Ashcroft, 1955; Fothergill & Yeoman, 1957; Fothergill & Jones, 1958; Fothergill & Hide, 1962) indicated that for some parasitic fungi the balance of the major inorganic salts in a culture medium was more important for the good growth of the mycelium than the concentration of individual salts, but that with some

saprophytic species the balance was much less important. For a wide range of fungi, however, the results varied. The object of the present experiments was to continue these investigations, with three species of the genus *Phytophthora* which are usually regarded as facultative parasites having only a short saprophytic existence in the soil. While a large amount of work has been done on these species, particularly *P. infestans* concerning taxonomy, cytology and host-parasite relationships, little comparative work seems to have been done on their mineral nutrition. Earlier experiments, such as those of Payette & Perrault (1944), were of small value from the nutritional aspect because of the use of undefined media. More exact investigations of these fungi were carried out by Hall (1959), Sakai (1955, 1956) and Mehrotra (1950).

METHODS

The following organisms were used: *Phytophthora infestans* (Mont.) de Bary, *P. erythroseptica* Pethybridge and *P. parasitica* Dastur. All of them were obtained from the Central Bureau voor Schimmelcultures, Baarn. *Phytophthora infestans* is the cause of Late Blight disease of potato; *P. erythroseptica* is a cause of Pink Rot of potatoes, 'shanking' in forced tulips, wilt in *Atropa belladonna* and other diseases; *P. parasitica* attacks *Ricinus communis* and causes 'damping off' and 'Foot Rot' of tomato and other plants. Throughout this paper the following abbreviations are used: *P. infestans* is designated PI; *P. erythroseptica*, PE, and *P. parasitica*, PP.

Initially single spore isolates of each of these species were prepared according to the method of Hall (1959) and stock cultures were maintained in 1 oz. screw-topped bottles each containing 10 ml. French bean oatmeal agar. The stock cultures were stored at 5° and subcultured at 3-monthly intervals, or when required. No alteration of morphological and cultural characteristics of these fungi occurred during the experimental period. In all experiments cultures were grown in 20 ml. medium in 150 ml. Erlenmeyer flasks. To avoid caramelization of media, sterilization was carried out at 115° for 10 min. After the growth period, the mycelia were filtered on to tared Whatman No. 5 filter-papers and washed with water. The mycelium with the filter paper was then dried overnight at 80–85°, cooled in a desiccator and weighed. Results are expressed as mg. dry wt. mycelium/flask, average of 4 or 5 replicates. It was not practicable to separate the mycelium from the filter paper after filtration, and, as the filter papers used lost 5% of their original weight on drying under the above conditions, appropriate allowances were made. For preliminary experiments the followed basal defined liquid medium A was used. This was a modification of a medium successfully used by Lopatecki & Newton (1956) for organisms PE and PP, in which the amounts of glucose and thiamine present were varied as follows: glucose, 25 g./l.; DL-asparagine, 4.0 g./l.; K₂HPO₄, 1.0 g./l.; MgSO₄·7H₂O, 0.1 g./l.; CaSO₄·2H₂O, 0.1 g./l.; FeSO₄·7H₂O, 0.001 g./l.; thiamine, 0.8 mg./l.; ZnSO₄·7H₂O, 1 p.p.m.; H₂MoO₄, CuSO₄·5H₂O and MnSO₄·4H₂O at 2 p.p.m. This solution was initially at pH 4.7.

Experiments were made to determine the best method of inoculation. It was found that a minced mycelium technique gave the best and most consistent results, giving a low coefficient of variation between replicate cultures and affording some control over the amount of fungal material added to each experimental flask. In this method, cultures were blended for 10 sec. with 50 ml. sterile distilled water in a

Monel metal container of an 'Atomix' blender. The resulting homogenate was then centrifuged for 5 min. at 3000 rev./min. in tubes with Oxoid metal caps; the supernatant liquid was discarded and the mycelium was taken up in sterile distilled water. This washing process was repeated three times. The mycelium was finally added to a known volume of sterile distilled water, 10 ml. removed and added to an EEL colorimeter tube and the reading recorded. By reference to a previously calibrated graph with a linear scale, the quantity of blended mycelium per ml. could be calculated and the volume of the remainder of the mycelial suspension adjusted to give a concentration of 1 mg. of blended mycelium/ml. water. One ml. of suspension obtained in this way was used as the inoculum in all subsequent experiments.

Preliminary experiments indicated that the optimum temperature for organisms PE and PP was 28°, and 20° for PI. These temperatures were used in subsequent experiments. PE grew faster at all temperatures than either PP or PI which had the slowest growth rate. With medium A maximum dry wt. mycelium was obtained with PE after 20 days of incubation, with PP after 13 days, and after 16 days with PI. These incubation times were used in subsequent experiments unless otherwise stated.

Further experiments with medium A showed that glucose, asparagine, KH_2PO_4 and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ were essential nutrients for all three fungi. The omission of $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ from the medium decreased the growth of PE by 70 %, of PI by 50 % and of PP by only 7 %. The omission of the trace elements had little effect on growth-yield of PP but seriously affected that of the other two species. Omission of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ had a very marked effect on the yield of PI and a considerable effect on PP and PE.

The growth of PE and PP in this medium resulted in an increase of acidity as great as 3.5 pH units. The optimum pH for both PP and PE was 6.6, that for PI was 5.0. Medium A was thus subsequently modified by omission of the initial concentration of KH_2PO_4 replacing it with Sorensen's salt adjusted to give pH 6.6 (i.e. with KH_2PO_4 , 4.746 g./l. + $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, 5.443 g./l.). This medium is referred to as medium B and was used subsequently for organisms PE and PP. Sorensen's salt at any concentration had a distinctly depressing effect on the growth of PI and medium A at pH 4.7 was used for this fungus.

Tests showed that none of the fungi needed an exogenous supply of biotin, pyridoxine, nicotinamide, folic acid, riboflavin, calcium pantothenate, *p*-aminobenzoic acid, inositol or ascorbic acid; but thiamine was necessary for good growth. These results confirm those of earlier workers (Robbins, 1937; Cantino, 1955). Preliminary experiments with a wide range of structurally different carbon compounds also showed that the best carbon sources for these fungi were those of similar constitution, namely, sucrose, glucose and mannose for organisms PP and PI, and sucrose, glucose and fructose for PE. Similarly the best nitrogen sources in order of mycelial yield were as follows: for PE glycine, calcium nitrate, cystine, ethyl aminoacetate hydrochloride, histidine, asparagine; for PP aspartic acid, glycine, calcium nitrate, asparagine, γ -aminobutyric acid, ethyl aminoacetate hydrochloride; and for PI γ -aminobutyric acid, citrulline, glutamine, arginine and asparagine.

RESULTS

Sulphur requirements

While Mehrotra (1950) made a survey of the sulphur requirements of some species of *Phytophthora*, the results were criticized by Cantino (1955) because of a lack of pH control during the growth period. The sulphur requirement of the *Phytophthora* species used in the present work was thus investigated by varying the sulphur sources individually in media A and B. The sulphur compounds were each added in turn to these media to give a final concentration of 0.03 g./l. The basal media were modified by the replacement of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and trace elements containing sulphur with $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ and $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ to give final concentrations of 0.1, 0.1 and 0.001 g./l., respectively. Preliminary experiments showed that the addition of chloride ion as KCl did not

Table 1. *The utilization of inorganic and organic sulphur compounds by Phytophthora species*

Highest yields expressed as percentages of yields from $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$ taken as 100. Initial pH value for *Phytophthora erythroseptica* (PE) and *P. parasitica* (PP) = 6.6; for *P. infestans* (PI) = 4.7. Final pH values recorded in table. (Mg. dry wt. mycelium/flask average of 5 replicates. Incubation period for PI 16 days; those for PE and PP shown in brackets.)

Sulphur source	Species					
	Organism PE (mg. dry wt./flask and incubation time)	Final pH value	Organism PP (mg. dry wt./flask and incubation time)	Final pH value	Organism PI (mg. dry wt./flask at 16 days)	Final pH value
Sodium sulphate	100 (20)	5.8	100 (17)	5.7	100	5.8
Potassium persulphate	75 (20)	5.8	100 (17)	5.5	24	3.9
Sodium metabisulphite	124 (25)	5.7	110 (17)	5.5	29	4.0
Sodium dithionite	100 (25)	5.1	90 (17)	5.3	33	4.2
Sodium thiosulphate	81 (20)	5.8	100 (17)	5.4	100	6.0
Sodium sulphite	124 (25)	5.8	132 (13)	5.3	81	5.8
L-cysteine hydrochloride	115 (25)	5.8	98 (17)	5.3	43	5.9
Cystine	86 (25)	5.7	93 (17)	5.6	95	5.1
Thiourea	27 (20)	5.9	28 (17)	5.4	57	7.3
Sodium sulphide	127 (25)	5.7	103 (17)	5.4	62	5.6
Sulphanilic acid	20 (20)	6.2	15 (13)	5.6	57	6.4
Sulphamic acid	26 (20)	5.8	14 (13)	5.8	43	4.1
Sulphanilamide	10 (25)	6.0	16 (13)	5.5	57	7.4
Methylsalicylic acid	18 (25)	6.1	22 (9)	5.5	19	3.9
Methionine	110 (25)	5.8	104 (17)	5.4	86	5.7
Thiazamide	20 (20)	6.2	17 (17)	6.0	53	7.5
Control (no sulphur)	17 (20)	6.1	16 (17)	5.5	24	4.7

affect the growth of these fungi. The modified medium A was inoculated with organism PI and the medium B with organisms PE and PP as previously described. Organisms PE and PP were incubated at 28° and PI at 20°. Dry weights of 5 replicate cultures were determined after 9, 13 and 17 days of incubation for PP, after 15, 20 and 25 days for PE, and after 16 days for PI. The final pH values of

the media were also determined. For organisms PE and PI there was a general increase in acidity varying between 0.4 and 1.5 pH units, while for PI the increase in acidity was never greater than 0.8 pH unit, but an increase in alkalinity as high as 2.8 pH units was sometimes recorded. The results are shown in Table 1 which records the highest percentage yields expressed in terms of the yield from $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$ as 100%. With $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$ the actual highest mycelial yields were 155 mg. after 17 days of incubation for organism PP, 94 mg. after 20 days for PE, and 21 mg after 16 days for PI.

Since persulphate breaks down to sulphate very easily in solution, the results for potassium persulphate must be treated with reserve. In general, the results indicated that sodium sulphate and sodium thiosulphate were very good sulphur sources for all three fungi, while sodium metabisulphite, sodium dithionite, sodium sulphite, sodium sulphide, cystine and methionine were good sources for organisms PE and PP, but gave only moderate or poor mycelial yields with organism PI. Essentially similar observations were made by Fothergill & Hide (1962) with four species of *Pythium*. Thus, in general, the oxidation level of the sulphur atom had no consistent significant effect on mycelial growth; a thiosulphate ($\text{S}_2\text{O}_3^{2-}$), a metabisulphite ($\text{S}_2\text{O}_5^{2-}$), a sulphite (SO_3^{2-}) and a sulphate (SO_4^{2-}) were all good sulphur sources. The yields from the sulphur-containing methionine and cystine were as high or nearly so, as the yields from the best inorganic sulphur sources in contrast to the findings of Volkonsky (1933) who worked with aquatic *Saprolegniales*. The other compounds used, particularly those containing a benzene ring (i.e. sulphanilic acid, thiazamide, sulphanilamide, sulphosalicylic acid), were very poor sulphur sources for all three fungi, except that moderate yields of mycelium were obtained with organism PI. These results are similar to those obtained by Steinberg (1941) with *Aspergillus niger*. No further correlation of structure with utilization could be made with the fungi used, thus emphasizing their ability to utilize a wide range of sulphur-containing compounds. Preliminary experiments indicated that the metabolism of organisms PE and PP, as expressed by their nutritional requirements, was very similar and differed considerably from that of organism PI. The results with the sulphur compounds have emphasized this distinction. Organism PI shows a much narrower range of sulphur utilization than organisms PE and PP, but it seems to possess an ability to use compounds which are not used to any great extent by the other two fungi.

The requirement for phosphorus

The importance of phosphorus in the growth of fungi is, of course, well known, but few comparative studies on the use of different phosphates by species of *Phytophthora* have appeared in the literature. Hence an experiment was done to study the effects of different phosphates on mycelial production. In this experiment medium A was used in which potassium dihydrogen orthophosphate was replaced by K_2SO_4 , 0.67 g./l. To this medium were added singly in turn various phosphorus compounds to give a final concentration of phosphorus 0.3 g./l. The medium was otherwise unbuffered at initial pH 4.7 and the final pH value, recorded in each case, showed an increase in alkalinity of 1.0–1.5 pH units. As before, dry weights of 5 replicate cultures were determined after 9, 13 and 17 days of incubation for organism PP, after 15, 20 and 25 days for PE, and after 16 days for PI. The results are shown in Table 2 which records the highest mycelial yields expressed as a percentage

of that for sodium dihydrogen orthophosphate taken as 100 %. With NaH_2PO_4 the actual highest mycelial yields were 148 mg. for organism PE after 25 days of incubation, 178 mg. for PP after 17 days, and 45 mg. for PI after 16 days.

For all organisms sodium dihydrogen orthophosphate, sodium metaphosphate and lecithin were excellent sources of phosphorus, while tetra-sodium pyrophosphate, sodium β -glycerophosphate and nucleic acid gave high yields with organisms PE and PP, but the corresponding yields for organism PI were greatly diminished. The highest comparative yields were obtained with lecithin for organism PE, and nucleic acid for organism PP but, as a whole, the inorganic phosphorus sources seemed to be better than the organic compounds. The remaining compounds gave poor yields of mycelium. There were not great differences in yield with favourable compounds but differences were noted in the rate of utilization of the compounds. For example, the two orthophosphates and sodium β -glycerophosphate were utilized much more rapidly than sodium metaphosphate or tetra-sodium pyrophosphate. Similar results have been recorded for species of *Pythium* by Hide (1961) and for *Rhizopus stolonifera* by Yeoman (1954). This suggests that orthophosphate is more readily available to these fungi and that metaphosphate and pyrophosphate are first converted in the mycelium to orthophosphate before they are utilized. Casein was a good source of phosphorus and gave visibly good mycelial growth but measurement was difficult because of heavy precipitation in the medium after incubation for 1 or 2 days; this precipitate was insoluble in water, N-HCl or H_2SO_4 and dry weights were not recorded.

Table 2. *The utilization of various phosphorus compounds by Phytophthora species*

Highest yields expressed as percentages of yields from $\text{Na}_2\text{H}_2\text{PO}_4$ taken as 100. Initially all pH 4.7. (Mg. dry wt. mycelium/flask average of 5 replicates.)

Phosphorus source	Organism		
	<i>P. erythroseptica</i> (mg. dry wt./flask and incubation time, days)	<i>P. parasitica</i> (mg. dry wt./flask and incubation time, days)	<i>P. infestans</i> (mg. dry wt./flask incubation time 16 days)
Sodium dihydrogen orthophosphate	100 (25)	100 (17)	100
Sodium metaphosphate	94 (25)	96 (17)	129
Tetra-sodium pyrophosphate	101 (25)	97 (18)	4
Sodium β -glycerophosphate	100 (20)	87 (18)	7
Sodium phosphite	8 (20)	19 (17)	4
Sodium hypophosphite	11 (20)	14 (18)	13
Nucleic acid	97 (20)	116 (17)	49
Lecithin	119 (20)	77 (18)	81
Casein	+	+	+
Control (no phosphate)	23 (20)	11 (18)	11

+, Good mycelial growth but weight not determinable.

Factorial experiments

Some of the basal physical and chemical requirements of a culture medium for the growth of the organisms PE and PP having been determined, the effect of the balance of the chemicals used in the medium was next investigated. The factorial design and statistical analysis of the results should indicate whether or not a balance

between the salts is necessary for high mycelial yields under the given experimental conditions. The direct effect of the individual salts in the medium and the interaction between them is also determined. Previous experiments showed that medium A was a satisfactory medium for organisms PE and PP but KH_2PO_4 was replaced by K_2HPO_4 at a concentration of 1.046 g./l. to give initial solutions nearer to the pH growth optimum for these fungi. The solution was adjusted to pH 6.6.

Initial factorial experiments showed that the highest concentrations of the salts were possibly limiting growth and this medium A was further modified to give basal concentrations of K_2HPO_4 at 1.568 g./l., of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ at 0.41 g./l. and of $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ at 0.414 g./l. The new solutions were also adjusted to pH 6.6. In all experiments the final pH values of all replicates were determined; the variation of pH value was never more than one pH unit. The cultures were incubated at 28° for 17 days for organisms PE and PP. The concentrations of the salts were fixed on the basis of halving and doubling those in the basal medium containing K_2HPO_4 . Thus K_2HPO_4 , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ were each used at three concentrations and all possible combinations of them were set up, giving a total of 27 variations. Each combination was done in quintuplicate, thus, with each species of fungus, 135 culture flasks in each experiment were incubated. Each 150 ml. Erlenmeyer flask contained 30 ml. medium. The results given are the average mg. dry wt./mycelium of 5 replicates. The grouped results are shown in Table 3 and the grouped analyses of variance are given in Table 4. For abbreviations of the salt concentrations used in the text below see Table 3.

Table 3. *Growth responses to two species of Phytophthora to different conditions of K_2HPO_4 , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$*

Concentration of K_2HPO_4 : P_1 , P_2 , P_3 , = 0.0045, 0.009, 0.018 g./l.; of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$: Mg_1 , Mg_2 , Mg_3 = 0.0008, 0.0016, 0.0032 g./l.; of $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$: Ca_1 , Ca_2 , Ca_3 = 0.0012, 0.0024, 0.0048 g./l.

Difference required between means for significance at odds of 99:1 for K_2HPO_4 , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ = 9.547 mg. for *P. erythroseptica* (PE) and 6.518 mg. for *P. parasitica* (PP).

K ₂ HPO ₄ and MgSO ₄ ·7H ₂ O conditions																	
P ₁						P ₂						P ₃					
Mg ₁		Mg ₂		Mg ₃		Mg ₁		Mg ₂		Mg ₃		Mg ₁		Mg ₂		Mg ₃	
PE	PP	PE	PP	PE	PP	PE	PP	PE	PP	PE	PP	PE	PP	PE	PP	PE	PP
Mean dry wt. mycelium/flask																	
Ca ₁	60	89	65	83	75	92	118	160	110	172	120	144	212	178	192	174	217
Ca ₂	56	100	66	90	70	92	109	172	117	149	81	154	177	172	149	191	169
Ca ₃	80	98	80	186	89	163	123	102	116	173	120	169	237	96	162	167	165

From the single salt analysis it is evident that there was no consistent result with all salts for both organisms. This analysis showed that the differences between mycelial dry weight with K_2HPO_4 were significant at all concentrations of the salt for both organisms, and in both cases the highest yield was obtained at the P_1 level of the salt. For $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ with both organisms the differences observed between the Mg_1 and Mg_2 levels of this salt were significant. For organism PE the highest mean weight was obtained at the Mg_1 level, while for organism PP the Mg_2 level

gave the highest yield. For $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ with organism PE there were significant differences between the mean mycelial weights at the Ca_1 and Ca_2 level and also at the Ca_2 and Ca_3 levels, but not between the Ca_1 and Ca_3 levels of this salt. For organism PE these latter levels gave the same mycelial yield but for organism PP the highest mean yield was obtained with the Ca_3 concentration.

Table 4. *Analysis of variance (grouped) for Phytophthora erythroseptica (PE) and P. parasitica (PP)*

Required 'F' and 't' values taken from Snedecor's tables (1946). Sums of squares and mean squares are omitted from table.

Variance	DF	F required odds			
		Found organisms		99:1	19:1
		organism PE	organism PP		
Total	134	—	—	—	—
$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$	2	20.12	2.53	4.88	3.11
K_2HPO_4	2	5419.19	325.55	4.88	3.11
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	2	64.66	60.61	4.88	3.11
$\text{CaSO}_4 \times \text{K}_2\text{HPO}_4$	4	25.48	50.99	3.56	2.49
$\text{K}_2\text{HPO}_4 \times \text{MgSO}_4$	4	94.25	72.41	3.56	2.49
$\text{CaSO}_4 \times \text{MgSO}_4$	4	4.54	36.73	3.56	2.49
$\text{K}_2\text{HPO}_4 \times \text{MgSO}_4 \times \text{CaSO}_4$	8	7.13	23.12	2.74	2.06
Residual error	108	—	—	—	—

The grouped analyses of variance showed that there were significant interactions between all the salts in the first order combinations for both organisms. These results show that there was a physiological balance in some cases between these salts under the given conditions. With organism PE the interaction between K_2HPO_4 and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ showed that at the P_1 and P_2 levels increasing the concentration of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ had only a small effect. The highest yield was obtained with the highest concentration of K_2HPO_4 and the lowest concentration of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; no balance is indicated. But with organism PP similar dry weights were obtained at the P_1 and P_3 levels with the Mg_1 level and, in general, increasing the concentration of K_2HPO_4 led to increasing yields relative to an increasing level of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; a balance between the salts is indicated. The interaction of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ is significant but there were only relatively small gross differences between the yields of PE. The highest yields were obtained with the Mg_1 and Ca_3 levels and with Mg_3 and Ca_1 levels. The Ca_2 levels did not show any consistent change in yield with increasing concentration of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$. With organism PP the interaction of these two salts is interesting. At the Ca_1 and Ca_2 levels, increasing the $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ concentration had little effect on yield and there were no significant differences between them, but with the Ca_3 level there appeared to be inhibition of growth with the lowest concentration of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$. Doubling the amount of this salt in the medium gave a great increase in mycelial yield which was maintained, but not improved, by further increase in concentration of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$. A balance between these two salts is necessary and seems to be operative in the ratio of 2 $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$:1 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$. With organism PE the interaction between K_2HPO_4 and $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ showed that at the P_1 , P_2 and P_3 levels there was increasing yield with increasing concentration of $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, but

at the P_1 level the highest yield was given with the Ca_3 level, while at the P_3 level it was given with the Ca_1 level. With organism PP at the Ca_3 level there was no consistent growth increase with increasing K_2HPO_4 concentration and the highest yield was obtained at the P_3 and Ca_2 levels. The yields were nearly identical at the P_1Ca_3 , P_2Ca_3 and P_3Ca_3 levels. Thus a balance is indicated.

The analysis of variance also showed that the interaction of all three salts was significant for both organisms. The concentration of K_2HPO_4 was here the most important factor controlling the yield of the organism PE. The highest yields were obtained at the P_3 level. At the P_1 level increasing the concentration of the other two salts only affected the yield slightly. At the P_3 and Ca_3 levels high yields were only obtained when the $MgSO_4 \cdot 7H_2O$ concentration was low. With organism PP there was little difference between the mean mycelial yields at the $P_2Mg_1Ca_2$ and $P_3Mg_2Ca_3$ levels. There was a consistently increasing yield as the concentration of the salts was increased proportionately from the $P_1Mg_2Ca_1$, $P_2Mg_2Ca_2$ and the $P_3Mg_3Ca_3$ levels. The highest mean weight of 191 mg. was obtained at the $P_3Mg_2Ca_2$ level, but weights of 186, 187 and 181 mg. were also obtained at the $P_3Mg_3Ca_1$, $P_3Mg_3Ca_2$ and $P_1Mg_2Ca_3$ levels, respectively, suggesting that the concentration is not so important when the other two salts are in a relatively high concentration in the approximate ratio of $2CaSO_4 \cdot 2H_2O : 1MgSO_4 \cdot 7H_2O$.

Balance between $MgSO_4 \cdot 7H_2O$ and $CaSO_4 \cdot 2H_2O$

The previous experiments indicated that there was a physiological balance between K_2HPO_4 and $CaSO_4 \cdot 2H_2O$. While balance has been shown to exist between various combinations of salts by different workers, this would seem to be the first time a balance has been indicated between these two salts. To confirm this result another experiment was designed to test the change in the amount of growth which might result when the concentrations of the salts were varied while still maintaining the balance between them. This experiment was designed factorially following the method of Talley & Blank (1941) and Fothergill & Ashcroft (1955). Thus the basic concentrations of $MgSO_4 \cdot 7H_2O$ and $CaSO_4 \cdot 2H_2O$ were 0.205 and 0.414 g./l., respectively; these were decreased to half in one set of solutions and increased twice and then four times in other solutions. This gave four treatments each having the same balance between the salts but the ratio of their concentrations was 0.5:1:2:4. Each of these four solutions was tested singly with K_2HPO_4 at 2.091, 3.136, 4.181 and 5.227 g./l., respectively, giving 16 solutions in all. The remaining ingredients of the basal medium remained the same. The medium was adjusted initially to pH 6.6 and the cultures incubated in 4-replicate at 28° for 11 days. The results expressed as mg. mean dry wt. mycelium per flask are shown in Table 5 where treatments are numbered 1-16 in parentheses.

The results showed that the mean mycelial yields for K_2HPO_4 at the P_1 , P_2 , P_3 and P_4 levels were not significantly different from each other but they were different from the yields at the Mg_1 and Ca_1 levels. To show the interaction of $MgSO_4 \cdot 7H_2O$ and $CaSO_4 \cdot 2H_2O$ 't' tests were performed. There were no significant differences between treatments 2, 3 and 4 at the P_1 level, but there was between treatments 1 and 2. At all other concentrations of K_2HPO_4 a similar pattern was shown, that is, there were no significant differences between treatments 6, 7 and 8, treatments 10, 11 and 12, and treatments 14, 15 and 16; but at the P_2 , P_3 and P_4 levels, respec-

tively, there were significant differences between treatments 14, 15 and 16. At the P_2 , P_3 and P_4 levels, respectively, there were significant differences between treatments 5 and 6, 9 and 10, 13 and 14 at these levels of K_2HPO_4 . These results confirm the conclusion that a balance between $MgSO_4 \cdot 7H_2O$ and $CaSO_4 \cdot 2H_2O$ exists. But since significantly different mean dry weights were recorded with the Mg_1 and Ca_1 levels at all levels of K_2HPO_4 , it is evident that the effect of the balance between $MgSO_4 \cdot 7H_2O$ and $CaSO_4 \cdot 2H_2O$ operated only after a certain concentration of the salts had been reached. Thereafter the concentration of either of these salts could be varied without affecting the mycelial yield of *Phytophthora parasitica*, provided that the concentration of the other salt is varied proportionately.

Table 5. *Mean dry weights and growth responses of Phytophthora parasitica after 11 days incubation at 28° on media with the same balance but with different concentrations of certain components*

Concentrations of K_2HPO_4 : P_1 , P_2 , P_3 , P_4 = 2.091, 3.136, 4.181, 5.227 g./l.; of $MgSO_4 \cdot 7H_2O$: Mg_1 , Mg_2 , Mg_3 , Mg_4 = 0.102, 0.205, 0.410, 1.620 g./l.; of $CaSO_4 \cdot 2H_2O$: Ca_1 , Ca_2 , Ca_3 , Ca_4 = 0.202, 0.414, 0.828, 1.656 g./l. 't' = 2.447 for 6 degrees of freedom at P = 0.05; values of 't' for combinations of treatments, 1×2 = 4.063, 2×3 = 0.390, 3×4 = 0.917, 5×6 = 7.170, 6×7 = 0.780, 7×8 = 1.764, 9×10 = 4.176, 10×11 = 0.258, 11×12 = 0.249, 13×14 = 17.159, 14×15 = 0.599, 15×16 = 0.532. Figures in brackets are treatment numbers, i.e. 1-16, referred to in text.

	Mg_1 Ca_1	Mg_2 Ca_2	Mg_3 Ca_3	Mg_4 Ca_4	Mean N
Mean dry wt. mycelium (mg./flask)					
P_1	114 (1)	152 (2)	154 (3)	140 (4)	140
P_2	97 (5)	173 (6)	162 (7)	143 (8)	143.7
P_3	106 (9)	141 (10)	144 (11)	146 (12)	134.2
P_4	92 (13)	166 (14)	157 (15)	147 (16)	140.5

General mean = 139.6 mg. dry wt. mycelium/flask.

CONCLUSION

In general, the results of the factorial experiments showed that there was a considerable difference between the nutritional requirements of *Phytophthora erythroseptica* (PE) and *P. parasitica* (PP). With the individual salts used in the medium the greatest effect on mycelial growth was produced by varying the concentration of K_2HPO_4 for both organisms. Results for the other two salts were more variable and significant differences in dry weights did not vary in any general way. An absolute balance between the major mineral nutrients studied was not shown for *P. erythroseptica* but was shown for all combinations of salts with *P. parasitica*. The latter fungus seems to have more specific nutritional requirements than the former. The interaction of $CaSO_4 \cdot 2H_2O$ and $MgSO_4 \cdot 7H_2O$ at higher concentrations was outstanding for *P. parasitica*. Although the omission of $CaSO_4 \cdot 2H_2O$ from the original medium A had only a small effect on growth, the increase in acidity of the unbuffered medium during growth was great. Hence the interactions of $CaSO_4 \cdot 2H_2O$ and $MgSO_4 \cdot 7H_2O$ may only occur at, or near to, the optimum growth pH for *P. parasitica*. Second-order interactions between three salts were demonstrated.

The results for *Phytophthora erythroseptica* and *P. parasitica* support the findings of other workers. For example, Talley & Blank (1941), Fothergill & Ashcroft (1955) and Fothergill & Hide (1962) found that a physiological balance between mineral salts in the medium was necessary for good growth of the fungal parasites *Phymatotrichum omnivorum* and *Venturia inaequalis*, and the two species *Pythium debaryanum* and *P. ultimum*. No balance, however, was required for *P. afertile* and *P. torulosum*. The highest mycelial yields (237 mg. for organism *P. erythroseptica* and 191 mg. for *P. parasitica*) were obtained with the modified liquid basal medium A containing glucose, 25 g./l.; DL-asparagine, 4.0 g./l.; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.001 g./l.; thiamine, 0.8 mg./l.; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 1 p.p.m.; H_2MoO_4 and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 2 p.p.m. with K_2HPO_4 , 0.018 g./l.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.0008 g./l.; and $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, 0.0048 g./l. for organism PE and K_2HPO_4 , 0.018 g./l.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.0016 g./l., and $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, 0.0024 g./l. for *P. parasitica*. *Phytophthora infestans* has the most exacting nutritional requirements but grows satisfactorily on fewer sulphur and phosphorus compounds than either *P. parasitica* or *P. erythroseptica*. Mycelial yields of *P. infestans* were also very much less than those for the other fungi for all variations tried of the two basal media A and B. The highest mycelial yield for *P. infestans* was only 58 mg. and was obtained with medium A above but containing K_2SO_4 , 0.67 g./l. and NaPO_3 , 0.3 g./l. in place of K_2HPO_4 .

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