CHANGES INDUCED BY TRAINING IN
PHYTOPHTHORA INFESTANS

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1. INTRODUCTION

WHEN newly obtained from the wild, strains of the fungus *Phytophthora infestans* belonging to the same physiological race differ in their ability to overcome the field resistance of commercial varieties of potato. These differences have been related to the varieties from which the strains were obtained. Thus a strain usually attacks the tubers and leaves of the variety from which it was obtained in the field more vigorously than the tubers and leaves of any other variety (Jeffrey, Jinks and Grindle, 1962). Whether this relationship is the result of selection, that is, the isolates recovered from a potato variety were those which, of all present in the atmosphere, were best able to attack that particular variety, or the result of adaptation, whereby an isolate having grown on a particular variety improves its ability to grow on that variety relative to all others, is not clear from the earlier experiments. In the present paper we shall describe experiments which assess the contribution of adaptation to the establishment of the specific relationship between strains of this fungus and the variety of potato from which they are obtained.

2. MATERIALS AND METHODS

All our strains of *P. infestans* were obtained from commercial potato varieties grown in the experimental field at Birmingham in 1958 and 1960. Their isolation, maintenance and properties have been described by Jeffrey *et al.* (1962). The strains will be referred to by the initial letters of the potato varieties from which they were obtained. For example, the isolates from Duke of York will be referred to as DY. Since only one isolate from each variety in each of the two seasons will be used in the experiments we will distinguish between them by following these initial letters by the numbers 58 or 60, for the 1958 and 1960 isolate, respectively.

All isolates have been maintained on slopes of chick pea agar in boiling tubes at 17 to 19° C. since they were first purified and subcultures only have been grown on other, specified materials during the course of the experiments.

During the course of these and earlier experiments (Jeffrey *et al.*, 1962) a number of characteristics of the strains of the fungus have been scored in order to assess their ability to attack various varieties of potato. The most important are :

1. The amount of growth of the fungus along the length of the cut surface of a half potato tuber over a period of 10 days at 17 to 19° C. and 100 per cent. humidity.

2. The number of days for a zoospore suspension placed on a detached potato leaf to sporulate at 17 to 19° C., 100 per cent. humidity and controlled, artificial illumination.

Other characters which have been recorded on occasions include :—

3. The lag period before any growth of the fungus is visible after the inoculation of potato tubers.

4. The proportion of inoculated tubers and leaves in which the fungus subsequently grows or sporulates, respectively.

3. RESULTS

(i) *Short-term training*

In short-term training the fungus is grown for one transfer only, under the conditions which it is hoped will induce a change in behaviour, before the effects of the treatment are assessed. A large number of experiments have been carried out involving short-term training. They fall into two categories.

A. Attempts to raise the level of performance of strains of the fungus on the leaves and tubers of the host plant after the fungus has been kept for some time on the chick pea medium.

B. Attempts to improve the performance of a strain on one particular variety of potato (other than the one from which it was obtained in the field) relative to that on all others: that is, to transfer the ability of a strain to grow best on the variety from which it was obtained in the field to a different variety.

Since only limited success has been obtained in the first category (A) and no success with the second (B) we shall describe briefly only one of the many experiments of each kind which have been carried out.

A. As a routine procedure all experiments involving the testing of some property of the fungus when grown on potato tubers or leaves have been designed so as to detect any effect of short-term training. Each experiment is first carried out using inocula obtained from the fungus growing on the chick pea medium. On completion, it is repeated immediately and identically except that each tuber or leaf in the second experiment is inoculated with fungus obtained from the corresponding tuber or leaf in the first experiment. Clearly, if growth on leaves or tubers of the host plant improves the ability of the fungus to grow on leaves or tubers, the fungus should give a better performance in the second than in the first experiment.

In a typical experiment nine 1958 isolates of the fungus growing on the chick pea medium were each inoculated in duplicate on to tubers of three varieties of potato and their rate of growth was determined (experiment 1). This was repeated immediately, inoculating each tuber used in the second experiment with inocula from the same variety of tuber in the first experiment (experiment 2). The mean rate of growth (mm./10 days) of the nine isolates on each of the three kinds of tuber, namely, Majestic, Arran Consul and the isolates "Own" variety in the two experiments are given in table 1. While these results show the now familiar superior growth of the isolates on tubers of their "own" variety, they provide no evidence

of a superior performance in experiment 2 and hence no evidence of a beneficial effect of training. An analysis of variance confirms the absence of any differences between the rates of growth of the isolates in the two experiments. This is the general conclusion from all comparisons of this kind made with recently isolated strains of the fungus.

With strains of the fungus which have been in culture for some time a significant improvement can sometimes be detected between the 1st and 2nd experiments. For examples, when the strains used

TABLE 1

The mean rate of growth (mm./10 days) of nine isolates on three kinds of potato tuber before (expt. 1) and after (expt. 2) short term training on potato tubers

	Majestic	Arran Consul	Own	Mean
Expt. 1 . .	24.43	25.19	29.25	26.29
Expt. 2 . .	22.68	26.13	30.81	26.54
Mean . .	23.56	25.66	30.03	

in the earlier experiment were one year older they showed a much reduced, but equal, rate of growth on their "own" and on Arran Consul tubers of 16.7 mm./10 days when the latter were inoculated from the chick pea medium. In the second part of the experiment, when the tubers were inoculated from other tubers of the same variety, the rates of growth were significantly higher at 22.7 and 18.5 mm./10 days on their "own" and Arran Consul tubers, respectively. Short-term training can therefore recover some of the loss in vigour which occurs among older strains of the fungus kept on the chick pea medium. In the experiment just described, training on the isolates "own" varieties recovers 35 per cent. and on Arran Consul 10 per cent. of the loss in vigour which has occurred since the isolates were first assayed for their rate of growth on these tubers. The greater improvement in the strains following growth on their "own" varieties, which is apparent in these results, is repeatedly confirmed in experiments which we shall describe later.

B. The following experiment with three isolates GW 58, AC 58 and M 58 is typical of the many attempts to improve the performance of isolates on specific varieties of potato by short-term training on these varieties. Each isolate was transferred from the chick pea medium to tubers of Golden Wonder, Arran Consul and Majestic. Two weeks later a zoospore suspension was prepared from each isolate growing on each kind of tuber, making nine suspensions in all. Each of these suspensions was then used to inoculate leaves of Golden Wonder, Arran Consul and Majestic and the time taken for sporulation to occur on four leaves of each variety was recorded.

An analysis of variance of these data is given in table 2. This analysis shows that the isolates, GW 58, AC 58 and M 58 differ in the speed with which they sporulate on leaves after they have been trained identically. It also shows that the speed with which they sporulate depends on the variety of tuber on which they were previously grown and hence on the training they had received. Thus all three isolates sporulate fastest on the leaves after growing on Arran Consul tubers and slowest after growing on Golden Wonder tubers, the difference, on average, being almost three days. The three isolates, however, are not modified to the same extent by the training which

TABLE 2

Analysis of variance of the number of days to sporulation of three trained isolates on leaves of three different varieties of potato

Source of variation	(I×T×L) error				(I×T) error	
	N	MS	VR	P	VR	P
I. Isolates	2	1860.3	50.1	<0.001	10.1	0.05-0.01
T. Tubers used for training .	2	322.1	8.7	0.01	1.8	...
L. Leaves used for testing .	2	32.4	...	...	...	...
I×T	4	183.8	4.9	0.05-0.01	...	...
I×L	4	49.8	...	...	...	...
T×L	4	43.9	...	...	...	...
I×T×L	8	37.1	...	...	...	...

each received (I×T significant). On the other hand there is no evidence that after growing on tubers of a particular variety an isolate then sporulates faster on that variety than on the other two varieties. Indeed there is no indication from the analysis of variance that after growing on tubers of a particular variety an isolate sporulates faster on leaves of any one of the three varieties relative to the other two (T×L not significant). The result of short-term training in this experiment is general rather than specific; that is, while growth on tubers of different varieties alters the speed of sporulation to different extents (T significant) the extent of the alteration is the same on all three varieties of leaves.

(ii) Long-term training

It was reported earlier that repeated transfer on the chick pea medium leads to a progressively poorer performance of strains of *P. infestans* when subcultured on to tubers and leaves. In terms of the ability to attack the host plant the fungus degenerates, although in terms of its ability to grow on the chick pea medium it improves, at least initially (Jeffrey *et al.*, 1962). At the same time the ability of the fungus to grow and sporulate better on the variety of potato from which it was obtained is gradually lost. All strains eventually undergo these changes although to varying extents and at differing rates. This

is illustrated in fig. 1 for strains GW 58 and M 58 which show the greatest and least changes respectively as a result of their maintenance on the chick pea medium. It seems likely that the changes are the result of the method of maintenance and that they are an adaptive

FIG. 1.—The rates of growth of two strains of *P. infestans* (GW 58 and M 58) on tubers of their own variety and of Arran Consul measured at various times between October 1958 and December 1961. The two strains represent the extremes in behaviour. GW 58 shows a marked deterioration and M 58 almost no change with age. Both show a rapid loss of the ability to grow faster on their own variety.

response to the latter. If this is the case, we might expect to reverse them by suitable training such as long-term growth on the host plant. This expectation has been tested in the following experiments.

A. Training on tubers. Ten isolates were used in this and in all subsequent experiments; they were DY 58, M 58, GW 58, HG 58,

AC 58, DY 60, M 60, GW 60, HG 60 and AC 60. At the beginning of the experiment the 1958 isolates were three years old and the 1960 isolates one year old. Each strain was transferred in quadruplicate from the chick pea medium to tubers of two varieties of potato, the one from which it was originally obtained (the isolates "own" variety) and Arran Consul. For example strains DY 58 and DY 60 were transferred to four tubers of Duke of York and four of Arran Consul. The strains obtained from Arran Consul, that is AC 58 and AC 60, were transferred to eight tubers of Arran Consul, four of which were designated "own" throughout the course of the experiment and four as Arran Consul. The usual metrics, the number of successful transfers, the number of days before growth commenced (lag period) and the amount of growth in ten days, were recorded. After eleven days each strain growing on each tuber was transferred to a tuber of the same variety. Thus, DY 58 growing on Duke of York tubers was transferred to further Duke of York tubers and the same strain on Arran Consul tubers was transferred to further Arran Consul tubers. The other isolates were treated similarly. Where all four replicates grew each replicate in the second and subsequent transfers was made from the corresponding replicate in the previous transfer. Where one or more replicates failed to grow they were replaced at the next transfer by inocula from the replicates which did grow. Where all four replicates failed, as they did with some of the more degenerate 1958 isolates, the second transfer was made with inocula from the chick pea medium. If, however, all four replicates failed again no attempt was made to set up a third transfer from the chick pea medium. Apart from this the third and subsequent transfers were made in the same way as the second. Hence, the replicates were, as far as was practicable, independently maintained subcultures throughout the training period and the testing period which followed. The same three metrics were recorded following each transfer. Their values in 5 successive transfers on the tubers averaged over isolates and replicates are shown separately for the 1958 and 1960 strains in fig. 2.

Only two of the five 1958 isolates, namely DY 58 and M 58 survived the training period and they did so on both their "own" and Arran Consul tubers. But throughout the period of training their performance as measured by all three metrics showed a gradual deterioration which occurred equally on their "own" and Arran Consul tubers.

All of the 1960 isolates survived the training period on both their "own" and Arran Consul tubers. Furthermore on their "own" variety of tuber they showed a gradual, cumulative improvement in all their growth characteristics. On the Arran Consul tubers, however, there was no improvement and the performance of the strains remained virtually unaltered throughout the training period.

The conclusions to be drawn are clear. The 1958 isolates cannot

FIG. 2—The changes in growth characteristics in five successive transfers of five 1960 strains and the surviving 1958 strains on tubers of their own varieties and of Arran Consul. Each point on the graph is averaged over replicates and strains. The 1960 strains show a gradual improvement in all their characteristics on their own variety but not on Arran Consul. Hence the differences (OWN-AC) gradually change in favour of OWN. The surviving 1958 strains show a gradual deterioration for all characteristics regardless of the varieties of potato used. There are therefore no obvious trends in the (OWN-AC) differences.

be trained by the method we have used, whereas the 1960 isolates can, but only on their "own" variety of tuber.

B. Training on leaves. Using the same ten strains of *P. infestans* and experimental design as in the previous experiment each strain, in the form of a zoospore suspension, was placed on four leaves of its

FIG. 3.—The changes in days to sporulation in six successive transfers of five 1960 strains and of the surviving 1958 strains on leaves of their own variety and of Arran Consul. Each point on the graph is averaged over replicates and strains. There is little overall improvement in the 1960 strains but the advantage of these strains on their own variety (AC-OWN) gradually increases. The 1958 strains show an overall improvement on their own variety but not on Arran Consul.

"own" variety and of Arran Consul. After the time of sporulation had been recorded a zoospore suspension was prepared from each infected leaf and this was used to inoculate a further leaf of the same variety. Where an inoculated leaf produced no sporulation a replicate was used to provide the inoculum for the next transfer. Where no replicate was available an inoculum was obtained from the strain growing on the chick pea medium for the second but not for any subsequent transfer.

The change in the mean number of days to sporulation in successive transfers averaged over isolates and replicates is shown for the 1958 and 1960 isolates in fig. 3. Only two of the 1958 strains, namely, M 58 and HG 58 survived the period of training on both their "own" and Arran Consul leaves while DY 58 survived training on its "own" variety. Two of these strains, DY 58 and M 58, were the only ones

to survive the training on tubers. The initial response of the surviving strains to growth on the leaves was to show an increase in the time required for sporulation to occur on both their "own" and Arran Consul leaves. This was followed by a gradual reduction in the time to sporulation on both kinds of leaf, which was more marked on their "own" variety than on Arran Consul. Indeed at the end of the training period there had been no overall improvement on Arran Consul while on their "own" variety the time to sporulation was much less than that of the untrained strains.

The initial response of the 1960 isolates to growth on leaves is also to show an increase in the time taken for sporulation to occur. On Arran Consul, however, there is no subsequent recovery; on their "own" leaves there is, but it is late in onset and the overall improvement is slight. In consequence, while the 1960 isolates sporulate initially much faster than the surviving 1958 isolates, at the end of the training period the performance of both sets of isolates is virtually identical whether on their "own" or on Arran Consul leaves.

Our conclusions, therefore, are that the 1958 isolates have in some cases changed too much to be trained to attack leaves and those which can still be trained improve only on their "own" leaves. Since the 1960 isolates are capable of almost no improvement, their performance on leaves is presumably already close to the optimum. They deteriorate, however, on Arran Consul leaves. Thus, these results agree with those obtained from the previous training programme on tubers in indicating a special relationship between an isolate and its "own" variety of potato.

(iii) *General and specific changes*

The changes induced by training on tubers and leaves are specific in that they differ according to the variety of potato used. But how far does this specificity extend? For example, do strains trained on tubers show an improved performance on leaves and do strains whose performance has been improved by training on their "own" variety also show an improvement on other varieties? These possibilities were examined in the following series of experiments.

A. Strains trained on tubers. By the end of the training period the five 1960 strains and the two surviving 1958 strains were available in three forms:—

UT. Untrained, that is the strain on chick pea medium.

TO. Trained on their "own" variety of tuber.

TAC. Trained on Arran Consul tubers.

To compare the trained and untrained forms and to determine whether any changes resulting from the training were confined to growth on the variety of tuber used for training, the UT, TO and TAC forms of each isolate were inoculated on to tubers of both their "own" variety and Arran Consul. Four replicates of each treatment

were set up and the whole experiment was divided between two randomised blocks. The usual three metrics were recorded and their average values for the 1960 and 1958 strains in each of the six treatments, that is, for the UT, TO and TAC inocula grown on their "own" and Arran Consul tubers, are given in table 3. These results, in general, confirm those obtained earlier during the training period (fig. 2). For example, they show that the 1960 strains trained on their "own" tubers (TO) are superior to the same strains which have not been trained (UT) or have been trained on Arran Consul tubers (TAC). In fact the latter turn out to be inferior to the untrained

TABLE 3

The rates of growth (mm./10 days), percentage of successful inoculations and lag periods (days) of the three forms of each strain (UT = untrained, TO = trained on own tubers and TAC = trained on Arran Consul tubers) when grown on their own and Arran Consul tubers and averaged over all the 1960 strains and the surviving 1958 strains

Character	Rate of growth		Percentage success		Lag period	
Type of inoculum	Variety grown on					
	OWN	AC	OWN	AC	OWN	AC
1960 strains { UT TO TAC	22.50	23.95	95	90	6.05	6.35
	28.58	23.55	100	100	5.93	6.25
	14.05	17.65	75	75	8.20	7.75
1958 strains { UT TO TAC	23.38	23.13	88	100	7.25	6.78
	4.75	10.13	50	38	10.13	10.25
	6.68	7.50	50	50	10.00	9.63

strains. These results also show us something new, namely, that the improved growth of strains trained on their "own" varieties (TO) is confined to growth on their "own" variety, indeed they grow no better on Arran Consul tubers than do the untrained strains (UT). The improvement induced by training on their "own" variety is, therefore, specific.

The results in table 3 also confirm those obtained earlier (fig. 2) in showing that the surviving 1958 isolates have deteriorated, during the attempt to train them on tubers, relative to the untrained strains (UT). There is some suggestion that this deterioration has affected growth on Arran Consul tubers less than that on their "own" tubers but this is not consistent over the three metrics.

When newly isolated, the 1960 strains of *P. infestans* grew 5.1 mm./10 days faster on their "own" than on Arran Consul tubers. The untrained isolates (table 3 and fig. 2) grew equally well on both their "own" and on Arran Consul tubers. After training on their "own" tubers the strains grew 5.0 mm./10 days faster on the latter than on

Arran Consul tubers (table 3). Thus training, when effective, leads to a recovery of the performance which typifies newly isolated, vigorous strains. But this is all it achieves. No property which was not possessed by the newly isolated strains has emerged by training strains which have lost some of their initial vigour.

These conclusions are based on the average performance of all the 1960 strains. What of the performance of the individual strains? An analysis of variance of the rate of growth data for the individual strains is given in table 4. The breakdown is essentially that of table 2. We can recognise the same three main items: I, the difference between the five isolates for four degrees of freedom; T, the difference between the three forms of each strain; UT, TO and TAC for two degrees of

TABLE 4

Analysis of variance of total growth (mm./10 days) of five 1960 strains of P. infestans in three forms, UT, TO and TAC, growing on two classes of tuber, "own" and Arran Consul

Source of variation	N	MS	Replicate error		I × T error	
			VR	P	VR	P
I. Isolates . . .	4	595.8	8.3	<0.001	3.3	0.10-0.05
T. Type of training	2	278.1	3.9	0.05-0.01	1.5	>0.20
V. Variety of tuber	1	0.0				
I × T	8	179.5	2.5	0.05-0.01	...	...
I × V	4	10.0	...	...	...	...
T × V	2	50.1	...	...	...	...
I × T × V . . .	8	20.7	...	...	...	...
Replicates . . .	30	72.1	...	...	...	...

* Derived from homogeneous interactions sums of squares.

freedom; and V, the difference between the two classes of tuber, "own" and Arran Consul for one degree of freedom. We can also recognise the same first and second order interactions between the main effects. In addition we have a replicate mean square for thirty degrees of freedom based on the block differences. Whether we used the homogeneous interactions mean square ($I \times V$, $T \times V$ and $I \times T \times V$) or the replicate mean square as the error the same three items are significant, namely, the difference between the isolates (I), the difference between the three forms of each isolate (T) and the interaction between them ($I \times T$). It appears, therefore, that the response of the different 1960 strains to the training was not uniform. Three of the strains DY 60, M 60 and AC 60 in the untrained form (UT) grew on their "own" tubers almost as rapidly as they did when they were originally isolated. These, therefore, showed only a slight improvement after training. Another strain, GW 60, on the other hand, had virtually lost the ability to grow on tubers but regained it after training on its "own" variety. The remaining strain HG 60 actually deteriorated during training and hence it behaved like the

older, 1958 strains. Thus the different responses of the 1960 strains to training appear to be due to the different extents to which they had deteriorated before the onset of the training programme.

It is significant that the only two 1958 strains to survive the training, DY 58 and M 58, came from the same varieties as two of the more vigorous strains isolated in 1960, DY 60 and M 60, and that the first 1958 strain to fail in the training programme, HG 58, came from the same variety, namely, Home Guard, as the poorest 1960 strain HG 60.

To test further the specificity of the changes induced by training, the 1960 strains which had been trained on tubers were scored for their speed of sporulation on leaves. The design was identical with that used in the previous experiment, the three forms of each strain,

TABLE 5

The number of days to sporulation of the three forms of each 1960 strain (UT, TO and TAC) when tested on leaves of their own variety and of Arran Consul, averaged over the strains

Type of inoculum	Variety tested on	
	OWN	AC
UT	6.4	7.1
TO	5.2	6.0
TAC	4.9	5.7

UT, TO and TAC as zoospore suspensions being placed on leaves of both their "own" variety and Arran Consul. The whole experiment was again divided into two replicate blocks. The average performance of the five isolates in each of the six treatments is given in table 5.

These results show that training, whether on the strain's "own" or on Arran Consul tubers, has significantly ($P < 0.001$) increased the speed of sporulation on leaves. This improvement, however, is non-specific in two senses. First, it has occurred in both the TO and TAC strains and while the improvement is greater following TAC training it is not significantly so. Second, the untrained strains (UT) sporulate 0.7 days earlier on their "own" than on Arran Consul leaves and this difference has neither increased nor decreased following training on their "own" or on Arran Consul tubers. Training on tubers, therefore, has a general beneficial effect on the ability of the 1960 strains to sporulate on leaves. This is in marked contrast to the specific effect it has on the growth on tubers.

An analysis of variance of the number of days to sporulation for the individual strains with an identical breakdown to that in table 4 shows a significant interaction between the different isolates and their past training, that is, UT, TO and TAC. This, of course, is

exactly as was found for the growth of these strains on tubers and the explanation is presumably the same, namely, that the strains have deteriorated to different extents prior to the training and hence improve to different extents as a result of the training. In fact the differences between the strains for rate of growth on tubers and for speed of sporulation on leaves are highly correlated though, of course negatively so ($r = -0.82$), because a good performance on tubers is represented by a high score but on leaves by a low score.

B. Strains trained on leaves. At the end of the training programme on leaves, each of the five 1960 strains and the two surviving 1958,

TABLE 6

The number of days to sporulation of the three forms of each strain (UT = untrained, LO = trained on own leaves, LAC = trained on Arran Consul leaves) when tested on their own and Arran Consul leaves and averaged over all the 1960 strains and the surviving 1958 strains

Type of inoculum	1960 strains		1958 strains	
	Variety tested on			
	OWN	AC	OWN	AC
UT	4.35	4.60	6.50	6.75
LO	3.30	3.95	3.25	4.38
LAC	5.15	5.30	4.00	5.38

strains were available in three forms which are comparable with those obtaining following training on tubers. The three forms will be referred to as,

UT, untrained, that is the stock cultures kept on the chick pea medium.

LO, trained on leaves of their "own" variety.

LAC, trained on leaves of Arran Consul.

Following the same procedure used for the strains trained on tubers, the three forms of each strain were scored for their speed of sporulation on leaves of their "own" variety and of Arran Consul. The average scores of the 1960 and 1958 strains for each of the six treatments are given in table 6. These show a small but significant ($P < 0.001$) reduction in the time taken for the 1960 strains to sporulate following training on their "own" leaves (LO) and an increase in this time following training on Arran Consul leaves (LAC). In these two respects the outcome is identical with the effect of training on the corresponding varieties of tuber on the subsequent growth on these tubers. They differ, however, in that training the 1960 strains on

their "own" leaves (LO) has produced an improvement in their speed of sporulation on Arran Consul leaves. This improvement is nevertheless smaller (table 5) but not significantly smaller than that on their "own" leaves. There is, therefore, some measure of specificity in the improvements induced by training on leaves but the specificity is less marked than that following training on tubers.

For the surviving 1958 strains the improvement following training on leaves of both their "own" variety and of Arran Consul is quite remarkable although not unexpected in view of the changes observed during the training period (fig. 3). The improvement is more marked when the training has been on their "own" variety and training on both varieties produces more improvement in the performance on their "own" than on Arran Consul leaves.

TABLE 7

The rates of growth (mm./days) of the three forms of each of the 1960 strains (UT, LO and LAC) when grown on their own and Arran Consul tubers, averaged over strains

Type of inoculum	Variety tested on	
	OWN	AC
UT	11.25	6.00
LO	19.45	13.30
LAC	10.95	12.25

An analysis of variance of these results, of the kind shown in table 4, indicates that the individual isolates are not showing the same response to training ($P < 0.001$). Two isolates, DY 60 and GW 60, show almost no improvement while the other isolates show considerable improvement after training on their "own" leaves.

To test further the consequences of training the 1960 strains on leaves the three forms of each strain, UT, LO and LAC were tested for their rate of growth on tubers of the strains "own" varieties and of Arran Consul in an experiment of the usual design. The mean performance of the five strains in each of the six treatments is given in table 7. These values are low compared with all those reported earlier for the growth of the same strains on the same varieties of tuber. This is an unavoidable consequence of the seasonal variation in the condition of the tubers and in their suitability for supporting the growth of the fungus. Experiments which, like those under discussion, extend over a long period always encounter this difficulty. However, while this makes comparisons between experiments conducted at different times unreliable it causes little difficulty where, as in the present case, our primary concern is in the relative performances

of the six treatments in the same experiment. This problem also arises with experiments on leaves but to a much lesser extent in that fresh leaves can be produced readily all the year round. The relative values of the six treatments in table 7 show that, in general, training on leaves has led to an increase in the rate of growth on tubers. Although the strains grow faster on their "own" tubers after they have been trained on their "own" leaves the improvement in growth on their "own" and Arran Consul tubers is almost equal. After training on Arran Consul leaves, on the other hand, the only improvement is in the rate of growth on Arran Consul tubers. This is the first and only indication of a specific improvement following training on a variety other than the strain's "own".

An analysis of variance of the results shows the usual pattern, namely, that the improvement following training varies from one strain to another. It also shows that the rate of growth on the two kinds of tubers depends on the previous training to which the strains have been subjected. The reason is presumably the one we have already noted, that is, following training on their "own" leaves (LO) the strains grow faster on their "own" tubers while following training on Arran Consul leaves they grow faster on Arran Consul tubers (table 7).

4. DISCUSSION

A. *The induced changes*

The changes in rate of growth on tubers and in speed of sporulation on leaves resulting from the training programmes are summarised in table 8. These are derived from tables 3, 5, 6 and 7 by subtracting the performance of the untrained strains from the corresponding performance of the strains following training on their "own" variety and on Arran Consul.

The improvement shown by the 1960 strains in rate of growth on tubers following training on tubers and in speed of sporulation on leaves following training on leaves, are specific on two counts (table 8). Firstly, an improvement occurs only if the training is on the strains "own" variety. Secondly, this improvement is confined to, or more marked on, the strains "own" variety than on Arran Consul.

In contrast the changes in rate of growth on tubers following training on leaves or in speed of sporulation on leaves following training on tubers are general rather than specific. Thus apart from the absence of an improvement on the strains "own" variety of tuber following training on Arran Consul leaves there is no further indication of a differential effect of the variety used for either the training or the testing.

The changes produced in the 1958 strains by training are also general rather than specific. They do, however, show a novel specificity in that the training on leaves produces an all round improvement while training on tubers produces an all round deterioration.

B. Conclusions

A number of conclusions may be drawn from our results. The first is that strains of the fungus which have lost some of their original properties while cultured on the chick pea medium may have these properties restored by appropriate training on the host plant. There are limits, however, to this recovery. Strains which have been in culture for three years (1958 strains) are incapable of improvement by training on tubers and only two show an improvement following training on leaves. Indeed, even after one year in culture (1960 strains) one of the strains is apparently beyond improvement by training on tubers. Thus the changes which occur in the fungus during the first year on the chick pea medium are, in general, reversible by our training procedures while those which occur principally between the first and third year are, in general, irreversible by the same treatments.

Our second conclusion is that any changes produced by training arise slowly and increase with the duration of the training. Often, no change can be detected after a single transfer on the host plant (section 3 (i)) and it usually takes three or more successive transfers to produce the maximum changes which have been observed (figs. 2 and 3). In fact it takes almost half as many transfers on the host plant to restore the original properties of the fungus as it took on the chick pea medium to produce their initial loss.

The third conclusion is that no property which was not possessed by the original strains is acquired by them as a result of training. That is, no strain grows faster on tubers of a particular variety or sporulates faster on the leaves of a particular variety at the end of the training period than when it was freshly isolated. Furthermore, there was almost no change in specificity induced by training. Thus the continual growth and sporulation of the strains on Arran Consul did not lead to superior growth and sporulation on this variety to that on their "own" varieties. In fact the best growth and sporulation achieved on Arran Consul was usually induced by training the strains on their "own" varieties.

The fourth conclusion is that training on leaves is more effective than training on tubers for the more degenerate strains. Thus, whereas three of the 1958 strains survived training on leaves and showed an improvement as a result, only two survived training on tubers and these were more degenerate at the end than at the beginning of the training period (table 8).

What may we infer from these conclusions about the nature of the changes induced by training? Our evidence consistently suggests that where training is effective it leads to the recovery of lost properties rather than the induction of new ones. The phenotype of the successfully trained strain is virtually identical with that when it was first isolated from the field. This loss and subsequent recovery of the initial phenotype involves either (a) a loss and recovery of the initial

activity of the genotype or, alternatively, (b) a loss and recovery of the initial genotype. The first of these interpretations requires that only non-heritable or extra-genic modifications are involved in the phenotypic changes restored by training. That is, the changes are comparable with enzymatic or cytoplasmic adaptations; the heritable potential remains unaltered and the substrate determines whether or not the potential is realised. On this interpretation the ability to respond to training is controlled by nuclear genes and the response

TABLE 8

The improvements in rate of growth on tubers and speed of sporulation on leaves following the training programmes on tubers and on leaves (summarised from tables 3, 5, 6 and 7)

Trained on :		Tubers				Leaves			
Tested on		Tubers		Leaves		Tubers		Leaves	
		OWN	AC	OWN	AC	OWN	AC	OWN	AC
Trained on									
1960 strains	OWN	6.1	-0.4	1.2	1.1	8.2	7.3	2.1	0.7
	AC	-8.5	-6.3	1.5	1.4	-0.3	6.3	-0.8	-0.7
1958 strains	OWN	-18.6	-13.0	...	...	...	...	3.3	2.4
	AC	-16.7	-15.6	...	...	...	...	2.3	1.4

is induced by exposure to the host plant. The inducer, however, is specific. A strain's "own" variety will bring about the restoration but Arran Consul (except where this is the strain's "own" variety) will not. Since exposure to a particular variety of potato will act as an inducer for one strain but not for another it follows that the heritable potential of strains isolated from different varieties must differ.

Some strains, particularly the older ones, cannot be restored to their original phenotype by training on the host plant. Since the inducers used with the older strains were the same as those which were successful with the corresponding younger strains we must conclude that the genotypic components have become defective in the older strains. That is, they have mutated to a non-inducible form.

The alternative explanation (b) assumes that the initial loss of properties results from genotypic changes, that is gene mutation, and the recovery of these properties during training results from backmutation. On this interpretation, strains which do not respond to training are presumably those in which many gene mutations have occurred during their initial run down. A particularly difficult fact

to explain on this interpretation is the specific effect of the variety of potato used on the outcome of training. If, as would seem likely, the pattern of mutation must be the same before, during and after the training period we must assume that the degeneration before training and the recovery during training is due to the selection of one type of mutant by the chick pea medium and a different type by the host plant. This assumption is reasonable in that any mutant which improves growth on the chick pea medium will be selected on this medium while one which improves growth on the host plant will be selected during the growth on the host plant. But this leaves unexplained the problem of why the mutants which have a superior growth on the host plant are apparently selected on one variety, their "own", but not on another variety, Arran Consul, even though they may have an advantage in growth on both varieties.

Irrespective of whether (a) or (b) is the correct explanation of the changes in the 1960 strains there seems little doubt that the 1958 strains have changed in a way that is not readily reversed and hence, presumably, involves one and possibly many gene mutations. If, as we have suggested in explanation (b), strains may deteriorate because of gene mutations and recover during training by the selection of backmutations it is surprising that tubers of their "own" variety and of Arran Consul are equally incapable of selecting mutants of the 1958 strains which have an improved growth on the host plant. It is also surprising, when we consider the results obtained with the 1960 strains, that in the two instances where 1958 strains recover by training on leaves the improvement takes place on both their "own" variety and on Arran Consul. If, before training, the 1958 and 1960 strains differ only in that the former have undergone more mutations than the latter then we should expect the 1958 strains to be less readily restored by training than the 1960 strains. But we should have expected some improvement in the 1958 strains whereas most of the strains showed none. Furthermore, we should not have expected the pattern of recovery in the 1958 strains, in which it occurs, to be so completely different from that observed in the 1960 strains. Clearly if we accept the behaviour of the 1958 strains as a characteristic consequence of gene mutations we can hardly explain the behaviour of the 1960 strains on the same basis. We must conclude, therefore, that the deterioration of the 1960 strains before training and the exact recovery of their initial phenotype during training are achieved without any gene mutations. Certainly there is no observation which we have made on the 1960 strains which cannot be explained in terms of enzymatic or cytoplasmic adaptation. In fact their behaviour differs from that of proven cases of adaptation in only one respect, namely, the slow rate at which the initial rundown and subsequent improvement take place.

It was concluded previously (Jeffrey *et al.*, 1962) that the differences between the strains obtained from different varieties of potato are

probably nuclear. That is, the ability of a strain from variety A to grow better on variety A than on variety B and of a strain from variety B to grow better on B than on A are probably genic in origin. If we now assume that the activities of these genes are inducible then the absence of the inducing agents in the chick pea medium and their presence in the strain's "own" variety will explain why the ability to grow faster on its "own" variety is lost on the medium and regained on the host plant. Furthermore, mutations of these genes in the older strains will explain their inability to respond to the inducer. And the occasional selection of a faster growing mutant of the older strains while growing on the host plant will explain their irregular, non-specific improvement.

If our interpretation of the training experiments is correct, the ability of a strain to grow better on its "own" variety than on any other variety of potato is induced and maintained by growth on its "own" variety. Thus the "nicking" we previously reported (Jeffrey *et al.*, 1962) between a strain and the variety from which it is isolated in the field has two components. Firstly, the ability of a potato variety to induce a particular strain to grow and sporulate at its maximum rate; this ability is presumably determined by the genotype of the host. Secondly, there is the ability of the strain to respond to the inducer which, as we have seen, is determined by the genotype of the strain. Hence, both selection of genotypic differences and adaptations induced by the host appear to play a part in the variation of *P. infestans*. But the role of adaptation is secondary; it merely uncovers potential variation which is already present in the population.

In the laboratory adaptive changes appear, from our experiments, to play a role in the loss of virulence of pathogenic fungi. And it is presumably the reversal of these changes by growing the fungus on the host which have led to the numerous claims that virulence can be induced by training (see Buxton, 1960 for review). By following the complete cycle of these events from the initial isolation to the recovery of virulence we have shown conclusively that no pathogenicity is gained by training which was not already present in the fungus at the time of isolation from the field.

5. SUMMARY

1. Short-term and long-term training has been carried out on strains of *P. infestans* of race 4 isolated in 1958 and 1960 and maintained on an artificial medium for three years and one year, respectively.

2. The training consisted of growing the strains on leaves and tubers of the variety of potato from which the strains were originally obtained in the field (their "own" variety) and on other varieties of potato, particularly Arran Consul.

3. The 1958 strains either failed to survive the training or deteriorated during the training. The 1960 strains improved as a result of long-term training on their own variety but not on Arran Consul.

4. The effect of training was specific in that the improvement induced by a strain's own variety was confined to growth on that variety. It was also general in that training on leaves produced an improvement on tubers, and vice-versa.

5. The improvements produced by training merely recovered the initial properties of the newly isolated strains which had been lost during their maintenance in the laboratory.

6. The changes in the 1960 strains which are reversed by training can be adequately explained in terms of specifically induced cytoplasmic adaptations. The failure of the more degenerate 1958 strains to respond to similar inducers suggests more stable nuclear changes.

7. Both selection of nuclear differences and cytoplasmic adaptation appear to play a role in the variation of *P. infestans* both in the field and in the laboratory. But the role of cytoplasmic adaptation is secondary.

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6. REFERENCES

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