JINKS, J. L. (1959). *J. gen. Microbiol.* 21, 397-409

Lethal Suppressive Cytoplasm in Aged Clones of *Aspergillus glaucus*

By J. L. JINKS

*A.R.C. Unit of Biometrical Genetics, Department of Genetics,
The University, Birmingham*

SUMMARY: An irreversible cessation of growth, accompanied by the death of the growing tips and the appearance of an intense brown pigment, is a regular feature of aged clones of *Aspergillus glaucus*. The frequency of this phenomenon, referred to as 'vegetative death', varies between clones of similar age and between identically maintained lineages of the same clone. It also varies with the method of propagation and with the other factors which affect the speed of ageing.

The heterokaryon and related tests show that vegetative death is cytoplasmic in origin while its segregation in the asexual progeny of colonies with mixed cytoplasm, and its infective spread via hyphal anastomoses, clearly define it as mutational in origin. Vegetative death involves a qualitatively different kind of cytoplasmic change from that previously found in the earlier stages of ageing.

In common with some other cytoplasmic mutants, those responsible for vegetative death suppress their wild type counterparts in cytoplasmic mixtures even though they are incapable of continued survival except in such mixtures. In this respect they differ from the generally reported behaviour of mixtures of mutant and wild type nuclei in heterokaryotic association. It is suggested, however, that the behaviour of either may be atypical, since most of the heterokaryons investigated will have been chosen for their stability, while a cytoplasmic mutant which was not suppressive is unlikely to be recognized and isolated by present techniques.

The phenotypic changes which occur in ageing clones of *Aspergillus glaucus* such as the decrease in rate of growth, pigmentation and perithecial production, have been explained in terms of reversible cytoplasmic changes resulting from readjustments in the equilibrium of normal cytoplasmic constituents (Jinks, 1954, 1956, 1957, 1958; Mather & Jinks, 1958). Sooner or later, however, in every ageing clone an irreversible cessation of growth occurs, which has been previously referred to as vegetative death. Its time of onset varies considerably; it has appeared in some lineages after as little as 5 months of vegetative maintenance while in others it has required as long as 2 years of similar maintenance. In the present paper investigations of vegetative death will be described which show that it results from a permanent heritable change in some cytoplasmic element and hence involves a qualitatively different kind of cytoplasmic change from that responsible for the earlier stages of ageing.

METHODS

The media and cultural conditions used were those described in the earlier papers. All the lineages and sub-clones described in this paper have been derived from the three original homokaryon clones 1, 2 and 4 of *Aspergillus glaucus* (Jinks, 1956) from which they differ by various introduced major gene mutations.

RESULTS

Description of vegetative death

The onset of vegetative death in a colony is marked by the death of only one or very few neighbouring growing tips. This leaves a gap in the otherwise circular growing front of the colony which widens into a sector as the remainder of the circumference continues to grow. This widening gap, however, is not invaded by the neighbouring, and as yet still growing, hyphal tips as would happen, for example, if a similar gap was made by the mechanical removal of a number of growing tips. On the contrary the cessation of growth spreads laterally from the initial sector to the neighbouring growing tips so that an increasing proportion of the circumference of the colony is involved. Estimates of the extent of vegetative death, measured as the percentage of the circumference which had ceased growing, are given for colonies from two ageing lineages of homokaryon 4 in Table 1. The lateral spread of vegetative

Table 1. *The percentage of the circumference of colonies of two ageing lineages of Aspergillus glaucus homokaryon 4 which had ceased growing, measured at weekly intervals*

Week	Lineage	
	4 ₁	4 ₂
	% of circumference which had ceased growing	
1	25	10
2	45	35
3	85	55
4	95	70

death within these colonies is shown by the steady increase in the percentages over the four weekly intervals. Unless large numbers of the unaffected tips are used to initiate new colonies as soon as the first dying sector is observed, the cessation of growth spreads to all the growing tips of the colony and further successful propagation is impossible. At this early stage it is still possible to obtain from these transfers some colonies which do not show further dying sectors. The majority of the transfers, however, give colonies which immediately produce multiple dying sectors and it becomes progressively more difficult to obtain from these sectors tips which are capable of even a limited amount of growth before they die (Pl. 1, figs. 1a, 2). This can be seen in Table 2 which summarizes the fate of transfers from a colony of lineage 4₁ (Table 1) when 55% of its circumference had already ceased growing. The transfers are divided into two sets according to whether they were taken from the vicinity of the dying sector or from the still growing part of the colony. Only one of the 6 transfers taken from the growing sector of the dying colony of lineage 4₁ gave a colony which produced no dying sectors and remained normal for the four weeks it was under observation. The colonies obtained from the remaining 12 transfers (Table 2) produced at least one dying sector and hence

ultimately died. However, by making early transfers from the least affected of these colonies, it was possible to obtain further colonies with dying sectors. Unfortunately, no more normal colonies were obtained by this means, but it kept the dying lineage alive while the analyses described later were performed.

Other symptoms were regularly associated with the cessation of growth in vegetative death. In every case so far examined there was an excretion of an intense brown pigment into the medium in the vicinity of the dying hyphae.

Characteristic microscopic symptoms are also associated with the death of the growing tips. Many tips are swollen to five times their normal diameter and their contents, which are initially dense, gradually disappear or are released onto the surrounding medium. At the same time the contents of older cells, some distance behind the growing tips, may disintegrate and the process spread towards the tips.

Table 2. *The number of dying sectors in 4-week old colonies obtained from hyphal transfers of the dying and still growing sectors of a colony of Aspergillus glaucus lineage 4₁*

Transfers from	Transfers tested (no.)	No. of colonies showing the following frequency of dying sectors			
		0	1	2	3 or more
Dying sector	7	0	1	1	5
Growing sector	9	1	3	5	0

Occasionally the cessation of growth is not final and a few tips may resume growth after being apparently dead for as long as 2 weeks. This new growth is sparse and mainly submerged; microscopically it appears normal. These recoveries, however, are short lived unless immediate transfers are made to new plates, where the growth is like that of an aged colony. Each such transfer produces multiple dying sectors within the first 2 weeks and no transfer has survived beyond 3 weeks.

Intra-clonal incidence of vegetative death

While vegetative death has never occurred spontaneously in other than severely aged material, all sister colonies of an aged lineage do not necessarily show vegetative death. Similarly, parallel lineages of the same aged sub-clone often differ in the incidence of vegetative death even when they are apparently equally affected by the other changes resulting from ageing. The most complete record of the incidence of vegetative death was provided by five parallel lineages within an ageing sub-clone of homokaryon 1₅. The lineages were initiated by choosing five colonies at random from the asexual progeny of a single colony. These five colonies and the lineages derived from them were cultured under identical conditions and propagated simultaneously, using the same type of inoculum. At any one time each lineage was represented by at least five sister colonies, and when it was necessary to obtain more detailed information the number was raised to twenty. The lineages were followed for a period of 6 months during which time eight successive propagations were

necessary. Although the colonies in the later stages of all five lineages showed the usual symptoms of ageing, in four of the lineages no colony showed vegetative death. In the fifth lineage, the later stages of which are summarized in Fig. 1, the first colony to show vegetative death appeared in the sixth propagation. In the seventh and eighth propagations 2 out of 19 and 6 out of 12 colonies respectively showed vegetative death. Thus the proportion steadily rose even though only hyphae from non-dying colonies were used in the propagation (Fig. 1).

Before the occurrence of vegetative death the fifth lineage was indistinguishable from the other four, even for rate of growth, which is the metric most sensitive to differences in ageing. But subsequent to the appearance of vegetative death in colony A2 in the sixth propagation, the mean rate of growth fell from 99.5 % to 79 % of that of the other lineages. Within the fifth lineage the same relationship held. Thus family D (Fig. 1) which alone showed no

Fig. 1. The fifth lineage of an ageing subclone of *Aspergillus glaucus* homokaryon 1_s from the 4th to 8th propagation. Colonies showing vegetative death are marked with a x.

vegetative death had a significantly higher rate of growth ($P = 0.02-0.01$) than the other colonies of the lineage even after omitting from the latter all colonies which were showing vegetative death at the time of scoring, namely, A2, A31, A32, A33, A34, B4, C11, C12 and E2. Thus a decrease in rate of growth was associated with vegetative death in the fifth lineage and indeed this decrease preceded the morphological symptoms.

More important, however, is the variation for these associated characters among colonies which have only recently been separated by vegetative propagations, and all of which originated from a single colony in the fourth propagation. From this it follows that either every case of vegetative death is an independent occurrence of the causal change or the causal agent must segregate among vegetative inocula taken from single colonies. Evidence for segregation is presented in the next and subsequent sections.

Method of propagation and intraclonal incidence

In so far as vegetative death has only been encountered in aged material, and indeed appeared to be an end product of the ageing process, all factors which affect the rate of ageing are prospectively important in determining the

frequency of vegetative death. Foremost among these factors is the type of inoculum used for propagation (Jinks, 1956). Results presented in the earlier paper amply illustrate these points; they do not, however, include investigations of the effect of method of propagation on colonies which are already showing vegetative death.

Since sexual reproduction is lost quite early in an ageing lineage it is only possible to compare the vegetative and asexual transfers of a colony which shows vegetative death. The transfers made from the asexual spores are initially without dying sectors and it may be some time before they appear. But since asexual spores are not produced in the immediate vicinity of the dying sectors those contained in a sample from a dying colony are obtained preponderantly from the more normal parts. Even so, very few asexual spores germinate, and attempts to improve their viability by the addition of casein hydrolysate (Jinks, 1958) failed. Still fewer asexual spores survive the early stages of growth and give rise to macroscopic colonies. While, however, the proportion of spores which germinate and give rise to macroscopic colonies is always low, the actual proportion varies with the extent of vegetative death in the parent colony. For example, between 2 and 7 % of the spores in random samples from dying colonies of homokaryon 1_s germinated and from 0.1 to 1.0 %, respectively, gave macroscopic colonies. The latter were superior to their parent colony and 97 % were without dying sectors after 3 weeks of incubation. Their appearance and rate of growth, however, was still that of colonies of an aged lineage, so that their superiority was prospectively short lived.

Vegetative transfers from the still growing portions of the same colonies of homokaryon 1_s showed between 70 and 100 % death in the first 2 weeks of growth. This performance is inferior to that of the colonies obtained from the asexual spore transfers. But it must be remembered that the spores which give rise to a colony are a highly selected sample of those produced by the dying colony. And presumably if a selection pressure between 1/100 and 1/1000 were practicable with vegetative transfers we might expect to obtain a similar proportion of normal colonies.

Further comparisons between vegetative and asexual propagations of single colonies showing vegetative death were made as follows. Hyphal tips of a dying colony which had ceased growth and which were incapable of further growth when transferred were traced back in the colony to the last head of asexual spores which they produced. Samples of single spores were taken from heads and attempts were made to germinate them and obtain macroscopic colonies. In a dying colony of homokaryon 4, 24 such heads were found which from their proximity to the dead hyphal tips must have been produced simultaneously with the cessation of growth. The results obtained are summarized in Table 3. In all, there were 7 viable spores from 4 heads; of these, 4 spores from 3 of the heads gave macroscopic colonies. And while all 4 colonies had the performance expected in an aged lineage only one showed vegetative death during the three weeks they were observed.

These results are virtually identical with those obtained in the previous

experiment. But in addition they show segregation within single heads of spores, each head having originated from a single cell. For example, of 50 spores taken from one of the 24 heads in Table 3, 47 were inviable, 2 gave colonies which soon showed vegetative death and one gave a normal macroscopic colony. Thus cell lineages which undoubtedly contain the causal agent of vegetative death can produce spores, albeit with a low frequency, which are apparently free from the agent or contain it at a much decreased concentration.

Table 3. *The distribution of viable spores, and of spores capable of giving a macroscopic colony, in 24 heads from a dying colony of homokaryon 4.*

Approximately 50 spores were taken from each head.

No. of heads sampled	No. of viable spores/sample	No. of macroscopic colonies produced
20	0	0
2	1	1
1	2	2
1	3	1
Totals: 24	7	4

The cytoplasmic basis of vegetative death

Heterokaryon tests. It was previously shown (Jinks, 1954, 1956) that an aged lineage whose colonies as yet showed no signs of vegetative death could be rejuvenated by heterokaryotic exchange of cytoplasm with a vigorous colony. As a consequence colonies of the rejuvenated lineage were still growing normally when those from the original aged lineage were showing vegetative death. At the stage when visible signs of vegetative death appear in the colonies the above situation no longer holds. And in every one of the numerous heterokaryotic combinations which have been made between dying and normal colonies the heterokaryon has behaved like the dying component, producing all the characteristic symptoms of vegetative death shortly after formation. (Pl. 1, figs. 1a, 1b).

The details of the analysis of these heterokaryons have varied with the available natural and introduced markers in the homokaryons in which vegetative death has occurred. But in all cases the criteria on which the nature of vegetative death has been decided have been the same as those summarized by Jinks (1958); the more important of these emerge in the following examples.

Lineage 4₁ in which vegetative death occurred carried a white asexual spore colour mutation. A heterokaryon was made between a dying colony of this lineage and a vigorous colony of lineage 4₂ which carried a buff asexual spore colour mutation. The asexual spores produced by the heterokaryon were sampled within 7 days of its formation, which was 2 days after it showed vegetative death. Less than 1 % of the spores in random samples germinated and these gave rise to homokaryon colonies with either white or buff spores and heterokaryon colonies with green, white and buff spores in the proportions 1:1.9:0.9, respectively. And although only the white-spored component

originally showed vegetative death, heterokaryon colonies and homokaryon colonies with buff spores were obtained which gave all the typical symptoms.

In a further analysis single heads of the buff and white homokaryotic spores produced by the heterokaryon were individually sampled. The results are given in Table 4. The total number of spores taken from each type of head was estimated by counting a small sample, while the number of viable spores and their subsequent performance was exactly determined. It is clear from Table 4 that irrespective of whether the genotype of the spores was that of the dying or vigorous component of the heterokaryon their performance was the same and like that of a dying colony. Furthermore, these samples gave colonies, with the genotype of the initially vigorous component, which showed vegetative death after up to 7 days of normal growth. These results provide strong evidence for the cytoplasmic origin of vegetative death in homokaryon 4; further proof for this particular case is given later.

Table 4. *The performance of asexual spores taken from single heads of buff and white homokaryotic spores produced by the heterokaryon*

	Performance		
	Non-viable	Viable	Showed vegetative death
Sum of 12 heads of white spores:	≈ 600	2	0
Sum of 11 heads of buff spores:	≈ 600	5	3

In other instances no appropriate colour marker was present in the lineage affected by vegetative death. Such a case arose in a lineage of homokaryon 1_g (Fig. 1) which had the wild type pigmentation. Derivatives of this homokaryon, however, readily form heterokaryons with homokaryon 2 (Pl. 1, figs. 1*a*, *b*) and both the homokaryons and the heterokaryons can be classified on the microscopic appearance of one or a few hyphae. As adult colonies they are readily distinguishable by colonial morphology, and spore colour differences are superfluous.

The viability of random samples of the asexual spores of the dying component of the heterokaryon and that of the asexual spores produced by the heterokaryon itself, were both between 4 and 5%. Without autonomous spore colour differences between the components of the heterokaryon, however, it is not possible to decide whether they are equally non-viable, though with such a low mean viability it is unlikely that any of the three types of spores produced by the heterokaryon have a normal viability. The viable fraction gave colonies of homokaryon 1_g and 2 both of which showed vegetative death with similar frequencies. The time of onset of vegetative death, however, was a little later in homokaryon 2 and the mean diameter of the colonies at death somewhat larger; this is shown in Table 5. Since some colonies die before measurements can be taken, and others are still growing normally some

time after their recovery from the heterokaryon, the results in Table 5 have been arbitrarily limited to those colonies which died between 3 and 24 days after germination. This difference in detail in the performance of colonies of homokaryons 1₅ and 2 recovered from the heterokaryon, does not detract from the proof of the cytoplasmic origin of vegetative death. Its origin presumably lies in the genotypic differences between the homokaryons, a subject considered later.

In all, four examples of vegetative death analysed by the heterokaryon test proved to be cytoplasmic in origin. These include examples in homokaryons 1₂ and 2₆ in addition to those just described.

Table 5. *The mean age and mean diameter of colonies of Aspergillus glaucus homokaryons 1₅ and 2, recovered from the heterokaryon, at the time of death.*

	No. scored	Mean age at onset of death (days)	Mean diameter (mm.)
Homokaryon 1 ₅	36	8.8	15.4
Homokaryon 2	22	10.0	28.6

Spread of vegetative death within colonies

A characteristic feature of a dying colony is the lateral spread of the cessation of growth to neighbouring growing hyphal tips (Table 1). Estimates of the speed of this spread in homokaryon 4₁ showed that the percentage of the circumference of the colonies which had ceased growth increased at the rate of 2.8 ± 0.3 % per day. When inocula containing dying hyphae are placed alongside normal hyphae of the same or of a different colony of the same lineage, in about 70 % of cases the normal hyphae show visible symptoms of vegetative death within 3 to 5 days. Furthermore, the site of the cessation of growth is not random but spreads outwards from the dying inoculum. In this way vegetative death can be induced in vigorous colonies of an unaged lineage of the same clone. For example it has been induced in colonies of the unaged lineage 4₄₄ (which has buff spores) on nine separate occasions by placing inocula from the aged lineage 4₁ (which has white spores) in their path. By varying the condition of the dying inocula used in these experiments it has been shown that hyphae which are affected by vegetative death but still capable of some growth must be present for successful induction. It would seem, therefore, that the appearance of vegetative death among neighbouring normal hyphae is the result of an infective spread from the affected hyphae.

In the induction of vegetative death in lineage 4₄₄ by lineage 4₁ the production of green heterokaryotic spores consistently preceded the cessation of growth in the neighbouring hyphae of 4₄₄. Furthermore, all the hyphae of 4₄₄ which died were formed after these spores were initiated. Also none of the numerous transfers of hyphae and spores produced by lineage 4₄₄ after their contact with lineage 4₁, but before the heterokaryotic spores were initiated, gave colonies which showed vegetative death. Yet similar transfers made at a later stage from the affected portions of 4₄₄ showed all the typical symptoms.

Such results leave no doubt that the infective spread of vegetative death within colonies is *via* anastomoses between affected and unaffected growing tips, with the ultimate death of the cell lineages which develop from the tips which receive the resulting mixed cytoplasm. Sharpe (1958) used the demonstration of infective spread as an alternative to the heterokaryon test for detecting cytoplasmic differences between two phenotypes; the criteria being the same in both tests. Since in the case of vegetative death the two methods have given identical results further details will not be given.

Reversal of vegetative death

Heterokaryons between dying and vigorous lineages of the same or different clones die soon after formation, but they do live somewhat longer, on the average, than their dying component in the homokaryotic state. Some temporary improvement is, therefore, introduced along with the cytoplasm of the vigorous component. This improvement can be maintained by continuously forming heterokaryons by using hyphae from a vigorous colony. In practice, the heterokaryon between dying lineage 4₁ and vigorous lineage 4₄₄ was surrounded by a dense ring of germinating asexual spores from 4₄₄. Heterokaryotic hyphae, recognized by their production of green spores, were isolated as soon as they appeared at the edge of this mass, and transferred to fresh plates. The procedure was then repeated on the longer-surviving transfers. At the third attempt two heterokaryotic colonies were obtained which produced no dying sectors during the 3 weeks they were under observation. The viability of their asexual spores was as high as 42 % and almost all gave adult colonies, irrespective of whether they had the white spores of lineage 4₁ or the buff spores of lineage 4₄₄. Three weeks after germination no homokaryon colony showed vegetative death and the re-extracted homokaryon colonies of 4₁ had a mean rate of growth of 1.53 mm./day compared with the last recorded rate before heterokaryosis of 0.91 mm./day. Thus by this technique colonies of lineage 4₁ were regularly recovered in a relatively unaged condition. The introduction of cytoplasm from the vigorous lineage is the essential part of this improvement but heavy selection for the more vigorous heterokaryotic hyphae is an inevitable result of the technique. However, controls involving the selection of the fastest growing tips of heterokaryons failed to produce colonies which did not show vegetative death.

The nuclear component of vegetative death

During the investigation of vegetative death some instances of nuclear participation emerged. In the first place the frequency of vegetative death varied significantly between identically maintained lineages of different genotypes. This had already been shown by the published frequencies of vegetative death for homokaryons 1, 3 and 6 (Jinks, 1956). It is, however, difficult to judge the importance of these observations in view of the magnitude of the variation in frequency of vegetative death between identically maintained lineages of the same genotype. A clearer example of nuclear

participation was given earlier in the present paper where it was shown that the time and stage of onset of vegetative death differed for the two components extracted from a dying heterokaryon.

The remaining example, now to be described, belongs to a different category of nuclear participation. It arose from attempts to induce vegetative death in unaged material by ultraviolet (u.v.) irradiation. Three examples were recovered following the irradiation of homokaryon 2₆ which showed all the visible symptoms of vegetative death (Pl. 1, fig. 8). In the one example analysed in detail, the appropriate tests failed to demonstrate a cytoplasmic component, and unlike our earlier examples of vegetative death, adding casein hydrolysate to the medium raised the viability of its asexual spores to normal. The subsequent behaviour of the colonies obtained from spores plated on the minimal medium and on minimal medium + casein hydrolysate when transferred to the minimal medium is shown in Table 6.

Table 6. *The behaviour of colonies of Aspergillus glaucus obtained from spores germinated on minimal medium or minimal medium + casein hydrolysate, when transferred to minimal medium*

Colonies from	Colonies tested (no.)	Performance of colonies on minimal medium		
		Normal	Dying sector	No growth
Minimal medium	26	21	5	0
Casein hydrolysate medium	20	8	4	8

The colonies with a normal phenotype and those which required casein hydrolysate for growth were true breeding, while those which produced dying sectors repeated the segregation of their parent colony (Table 6). Furthermore, the heterokaryon tests revealed only a nuclear difference between the two true-breeding segregants. It would seem, therefore, that the segregating colonies which show vegetative death must themselves be heterokaryons between the wild type segregant and its auxotrophic mutant. From this it follows that the vegetative death resulted from a breakdown of the heterokaryon to give homokaryotic sectors of the auxotrophic mutant which would have been incapable of prolonged growth on the minimal medium when no longer in close cytoplasmic contact with nuclei of the wild type component. This interpretation is supported by the increase up to 100 % in the proportion of spores with the genotype of the auxotrophic mutant, in the vicinity of the growing tips which have ceased growing.

DISCUSSION

These investigations have shown that spontaneous vegetative death in ageing lineages of *Aspergillus glaucus* is cytoplasmic in origin. But the properties of the cytoplasmic change which initiates vegetative death are unlike those responsible for the degeneration in sexual potential, rate of growth, etc. in ageing lineages. The latter involve no irreversible change in the structure or

function of cytoplasmic components and are explicable solely in terms of readjustments in the balance of normal cytoplasmic constituents. (Jinks, 1956, 1957, 1958). In contrast, the segregation from mixed cytoplasms of the cytoplasmic change responsible for vegetative death and its infective spread, both in the heterokaryon test and in its various modifications, clearly defines it as mutational in origin (Arlett, 1957; Jinks, 1958; Sharpe, 1958). Thus although ageing lineages regularly end in vegetative death the cytoplasmic change which produces the latter is not, apparently, a continuation of that responsible for ageing. On the other hand, it is difficult not to see a causal connexion between the two, whereby the degeneration of the cytoplasm, resulting from ageing, and the disorganization to which this presumably leads, increases its susceptibility to further breakdown.

It is also clear from the phenotypes of the colonies in the heterokaryon and related tests that in mixed cytoplasms the mutant responsible for vegetative death is suppressive in the sense defined by Darlington (1944). Thus the wild type cytoplasm is suppressed by the action or by the competitive reproduction of the cytoplasmic mutant. Suppression of one component of a mixed cytoplasm by the other appears to be the rule rather than the exception in the examples so far described (Ephrussi, Hottinguer & Roman, 1955; Pittenger, 1956; Arlett, 1957). Occasionally it has been the wild type component which has suppressed the mutant, but more often it has been the opposite. The present examples are particularly remarkable in that the suppressor is incapable of survival except when in mixtures with its wild type counterpart. As a consequence, dying sectors appear soon after these suppressive, yet lethal, cytoplasmic mutants have been introduced into a wild type cytoplasm either by mutation or by infection *via* hyphal anastomoses.

Suppression in mixed cytoplasms is in marked contrast to the behaviour of mutant and normal nuclear genes when contained in the same nucleus. Here the chromosome mechanism ensures that they are kept in step in reproduction and one cannot suppress the other because reproductively they do not compete. Competition at the cytoplasmic level presumably reflects the absence of comparable reproductive controls. A closer comparison might be expected with the behaviour of mutant and normal genes contained in different nuclei within a heterokaryon hypha; under these conditions the mutant and normal nuclei are free to compete in reproduction. In the majority of reported cases they establish equilibrium frequencies which are compatible with optimum growth (Beadle & Coonradt, 1944; Pontecorvo, 1946; Jinks, 1952; Rees & Jinks, 1952; Pontecorvo, 1958). Where optimal growth is obtained only in those hyphae where one component nucleus is in the homokaryotic state the heterokaryotic mixture breaks down. But it does so in favour of its competitively superior component. It is on this last point that the comparison between heterokaryotic associations and cytoplasmic mixtures has most frequently failed, in so far as the competitively superior component of the cytoplasmic mixtures has often been the poorer or even lethal phenotype in the pure state. This contrast, however, may be misleading. Few unstable heterokaryotic mixtures have been examined, if only because stability is a prerequisite

for the investigation in which they have been used. It was partly for this reason that the example of vegetative death which was produced by an induced nuclear mutation was given in detail. This example shows that nuclear mutations can be obtained which suppress their partners in heterokaryotic associations even on media where the suppressor is lethal in the homokaryotic state.

On the other side, the behaviour of the relatively few cytoplasmic mixtures described so far may well be atypical. And indeed it is difficult to see how the majority of cytoplasmic mutations would have been recovered if they had not suppressed their normal equivalents in the same or neighbouring hyphae. It seems probable therefore, that as methods of detecting and recovering cytoplasmic mutations improve, and more examples of unstable heterokaryons are analysed, the similarity between heterokaryotic associations and cytoplasmic mixtures will emerge as much greater than it appears at present.

I am indebted to Professor K. Mather, C.B.E., F.R.S., and Mr L. G. Wigan for helpful discussion during the preparation of this paper. I am also indebted to Miss B. Boldry for technical assistance.

REFERENCES

- ARLETT, C. F. (1957). Induction of cytoplasmic mutations in *Aspergillus nidulans*. *Nature, Lond.* **179**, 1250.
- BEADLE, G. W. & COONRADT, V. L. (1944). Heterocaryosis in *Neurospora crassa*. *Genetics*, **29**, 291.
- DARLINGTON, C. D. (1944). Heredity, Development and Infection. *Nature, Lond.* **154**, 164.
- EPHRUSSI, B., HOTTINGUER, H. M. & ROMAN, H. (1955). Suppressiveness: A new factor in the genetic determination of the synthesis of respiratory enzymes in yeast. *Proc. nat. Acad. Sci., Wash.* **41**, 1065.
- JINKS, J. L. (1952). Heterokaryosis: a system of adaptation in wild fungi. *Proc. Roy. Soc. B*, **140**, 83.
- JINKS, J. L. (1954). Somatic selection in fungi. *Nature, Lond.* **174**, 409.
- JINKS, J. L. (1956). Naturally occurring cytoplasmic changes in fungi. *C.R. Lab. Carlsberg (Série physiologique)* **26**, 183.
- JINKS, J. L. (1957). Selection for cytoplasmic differences. *Proc. Roy. Soc. B*, **146**, 527.
- JINKS, J. L. (1958). Cytoplasmic differentiation in fungi. *Proc. Roy. Soc. B*, **148**, 314.
- MATHER, K. & JINKS, J. L. (1958). Cytoplasm in sexual reproduction. *Nature, Lond.* **182**, 1188.
- PITTENGER, T. H. (1956). Synergism of two cytoplasmically inherited mutants of *Neurospora crassa*. *Proc. nat. Acad. Sci., Wash.* **38**, 442.
- PONTECORVO, G. (1946). Genetic systems based on heterocaryosis. *Cold Spr. Harb. Symp. quant. Biol.* **11**, 198.
- PONTECORVO, G. (1953). The genetics of *Aspergillus nidulans*. *Advanc. Genet.* **5**, 141.
- REES, H. & JINKS, J. L. (1952). The mechanism of variation in *Penicillium heterokaryons*. *Proc. Roy. Soc. B*, **140**, 100.
- SHARPE, H. (1958). A closed system of cytoplasmic variation in *Aspergillus glaucus*. *Proc. Roy. Soc. B*, **148**, 355.

J. L. JINKS—VEGETATIVE DEATH. PLATE 1

(Facing p. 409)

EXPLANATION OF PLATE 1

- Fig. 1*a*. Three colonies of the aged fifth lineage of homokaryon 1_s. The bottom two are showing the last stages of vegetative death.
- Fig. 1*b*. Three heterokaryons between the aged colonies of homokaryon 1_s shown in Fig. 1*a* and a vigorous colony of homokaryon 2_s. The phenotypes of the bottom two heterokaryons show the suppressive effect of the lethal cytoplasmic mutation responsible for vegetative death.
- Fig. 2. Examples of vegetative death in five different homokaryon clones. Three of the examples, i.e. the two on the left and the centre colony, arose spontaneously in aged lineages. The other two, on the right, were produced in vigorous lineages following heterokaryotic association with a colony showing vegetative death.
- Fig. 3. Four sister colonies, each obtained from single asexual spores of a colony which showed vegetative death after u.v.-irradiation. In this example the cause of vegetative death is an induced nuclear mutation requiring casein hydrolysate for normal growth and the symptoms are only shown on the minimal medium.

(Received 31 March 1959)