CHANGES IN THE HEAT-RESISTANCE OF ASCOSPORES OF NEUROSPORA UPON GERMINATION[1]

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ABSTRACT

LINGAPPA, YAMUNA, and A. S. SUSSMAN. (U. Michigan, Ann Arbor.) Changes in the heat-resistance of ascospores of Neurospora upon germination. Amer. Jour. Bot. 46(9): 671–678. Illus. 1959.—A rapid loss in heat-resistance accompanies activation of ascospores of *Neurospora tetrasperma* after incubation at 27°C. When activated spores are given a 5-min. "heat-flash" at 65°C. after only 5 min. at 27°C., fully ⅔ fail to germinate. Such treatment, if administered 25 min. after activation, results in the complete destruction of the spores. By contrast, when incubation at 27°C. is not interposed, more than ½ of the spores will germinate, even when they have been exposed to 65°C. for 30 min. Similar results were obtained with "heat-flashes" at 50 and 60°C., although exposures of longer duration were required to affect the spores. Conidia respond very differently to "heat-flashes" in that germination is stimulated if they are provided after an incubation period at 27°C. On the other hand, conidia are killed by short exposures to 60°C., so that they are far more susceptible to such treatment than are ascospores. A study of the cardinal temperatures of germination revealed that the maximum is about 44°C. for both conidia and ascospores. The maximum for the growth of two strains of *N. tetrasperma* and for one of *N. crassa* is between 40–45°C.; however, another strain of the latter species grows at 45°C. Dry heat was shown to be less effective than wet in activating ascospores. Removal of the exospore of ascospores results in the loss of considerable heat-resistance. In addition, the requirement for heat-activation is considerably mitigated in such spores, suggesting that the exospore, or an associated layer is the locus of the ascospore's heat-resistance.

IN MANY FUNGI, the conditions required for the germination of spores may be different from those that are optimal for prior, or subsequent, stages in development. This is shown very clearly when the cardinal temperatures for growth and germination are compared. Thus, Gäumann (1950) has reported that the spores of *Clasterosporium carpophilum* will germinate well between 9 and 27°C., whereas there is a pronounced optimum for growth at 22°C. Another striking illustration is that of the conidia of *Erysiphe graminis* which will germinate at 5–9°C., although growth of the germ tube is not supported at these temperatures. Reciprocally, at 25°C., germination is repressed but growth progresses well (Graf-Marin, 1934). In addition, there are several cases wherein the cardinal temperatures for the germination of different types of spores of the same organism may differ, as in some of the Uredinales (Doran, 1922; Arthur, 1929), *Pseudopeziza ribis* (Blodgett, 1935, 1936), and other organisms such as those discussed in the review by Sussman (1959).

The cases mentioned above involve only stages in the life-history of fungi in which clear morphological distinctions accompany the physiological ones revealed by the temperature optima. Yet, there are data which suggest that significant changes in metabolism may occur in certain fungal cells, even in the absence of morphological differences. Such an instance is that of the ascospores of *Neurospora* for which Goddard (1935) and Goddard and Smith (1938) suggested a qualitative change in metabolism after dormancy is broken. This suggestion has been supported by Sussman et al. (1956), Holton (1958) and Lingappa and Sussman (1959), who have shown that changes in the products of metabolism, endogenous substrates and content of cytochrome C mark the transition to the activated state in *Neurospora* spores. In addition, it is known that these cells require a heat-shock in order to germinate (Shear and Dodge, 1927; Goddard, 1935) and are very resistant to extremes of temperature (Faull, 1930). For these reasons, it appeared possible that the heat-resistance of these cells might change in response to activation and the following experiments were designed to test this suggestion.

MATERIALS AND METHODS.—One-year-old ascospores of *Neurospora tetrasperma*, assembled as described by Goddard (1935), were used throughout these studies. Conidia of strains 394.4 and 394.5, obtained through the kindness of Dr. B. O. Dodge, were subcultured on potato dextrose agar slants (PDA) and stored at 4°C. before they were crossed. The ascospores were washed repeatedly with distilled water, shaken in versene and washed as reported by Sussman (1954). Unless otherwise mentioned, the concentration of ascospores and conidia was standardized by the use of a blue filter (#44) in a Klett-Summerson colorimeter.

Germination of conidia was accomplished at first by suspending them in 0.01% potato-dextrose medium, but later it was found that the conidia of both *N. tetrasperma* and *N. crassa* could germinate equally well in distilled water. Activation of ascospores was accomplished by exposure to 60°C. for

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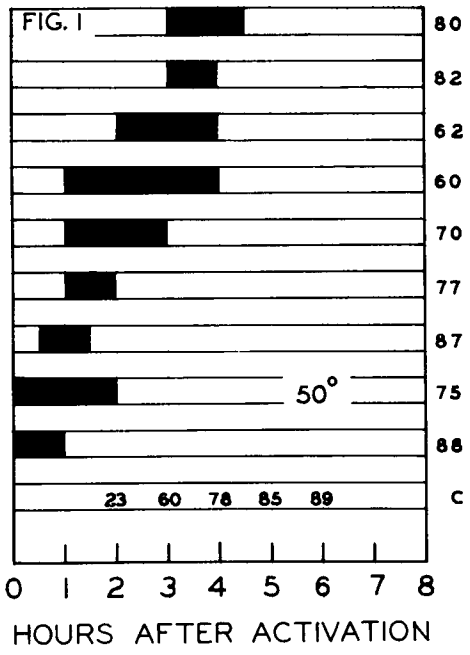
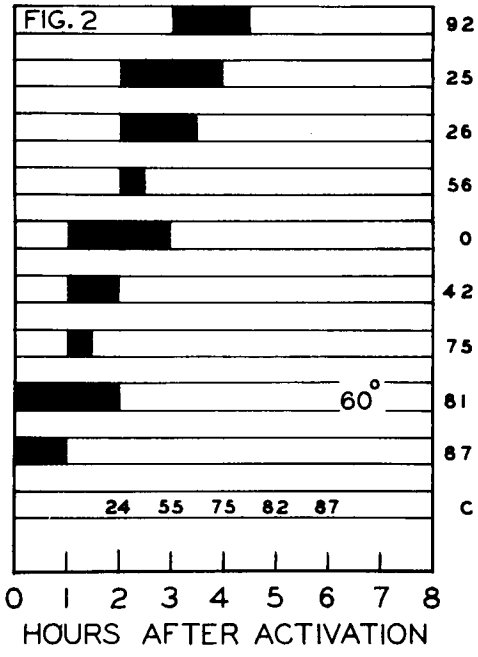
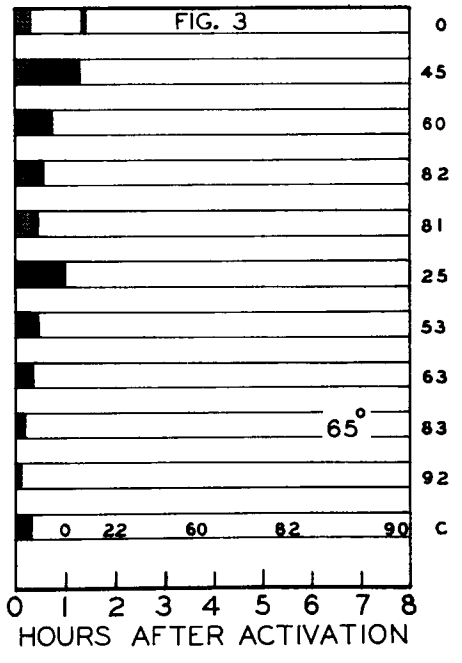






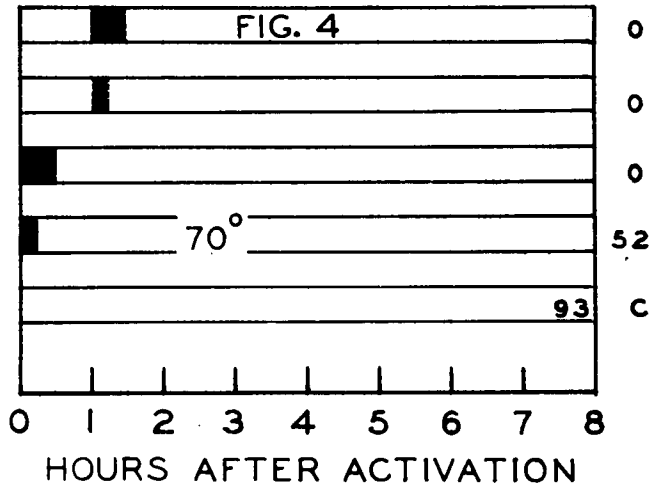


Fig. 1–4. Effect of exposure to high temperature upon germination of ascospores of *Neurospora tetrasperma*. Ascospores were activated at 60°C. for 20 min., before the start of the experiment, except for some of those used as outlined in fig. 3. Periods of incubation at 27°C. are indicated by the clear spaces and exposures to higher temperatures by the black ones. The letter *c* along the ordinate refers to the control which was incubated throughout at 27°C., and the numbers along this axis represent the percentage germination 8 hr. after the start of the incubation. The numbers within the control box are the germination percentages at the times indicated below on the abscissa.—Fig. 1. Heat-treatment at 50°C.—Fig. 2. Heat-treatment at 60°C.—Fig. 3. Heat-treatment at 65°C. The grey spaces indicate exposure to 60°C. for 20 min., and the black exposure to 65°C. No activation-treatment, other than treatment at 65°C., was provided the other spores used in this experiment. Exposures of 5, 10, 20, 30 and 60 min.—Fig. 4. Heat-treatment at 70°C.

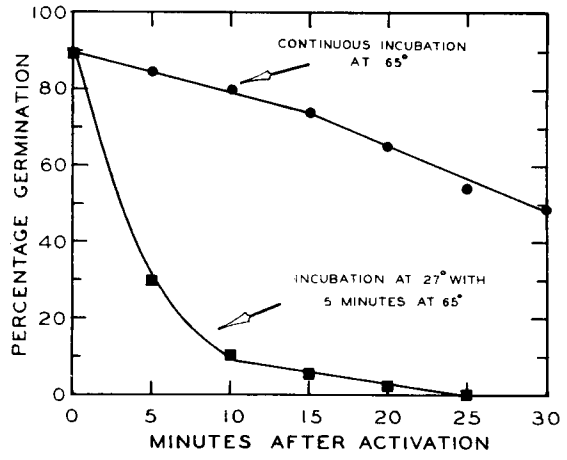


Fig. 5. Loss in heat-resistance of ascospores of *Neurospora tetrasperma* after brief incubation at 27°C. Points indicated by squares were derived from experiments wherein ascospores were activated at 60°C. for 20 min. and incubated at 27°C. for the times indicated on the abscissa, and exposed for 5 min. to 65°C. Thereupon, the spores were cooled and returned to 27°C., and incubated for a total of 4 hr. The points represented by circles were obtained from experiments in which ascospores were exposed to 65°C. immediately after activation, for periods of time from 5 to 30 min. Incubation at 27°C. was begun immediately after removal from the higher temperature, and the percentage of germination determined after 4 hr.

20 min. Treatment at other temperatures, after the activation period, was accomplished in a Dubnoff shaker in which the temperature was controlled to ±1°C. Incubation prior to germination was carried out on a rotary shaker maintained at 27°C. At the end of this incubation period, ascospores or conidia were killed by a few drops of commercial formalin. At least 600 spores, in duplicate samples, were counted in determining the percentage germination.

Dry-heat treatment was administered to about 2 mg. of air-dry spores in flasks incubated in the Dubnoff shaker. At the end of certain periods, they were cooled to room temperature with tap water, 6 ml. of water was added and incubation resumed at the specified temperature.

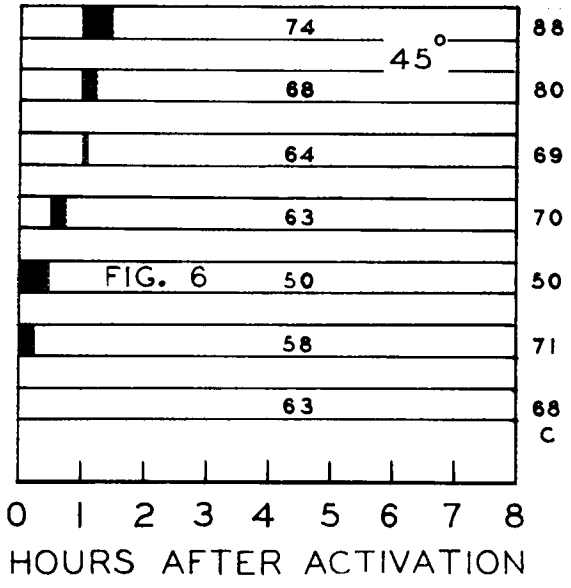
Removal of the ribbed coats (exospores) of ascospores was accomplished by the method described by Lowry and Sussman (1958) who used 10% commercial Clorox for 30 min. Hereafter, these spores will be referred to as "uncoated" spores.

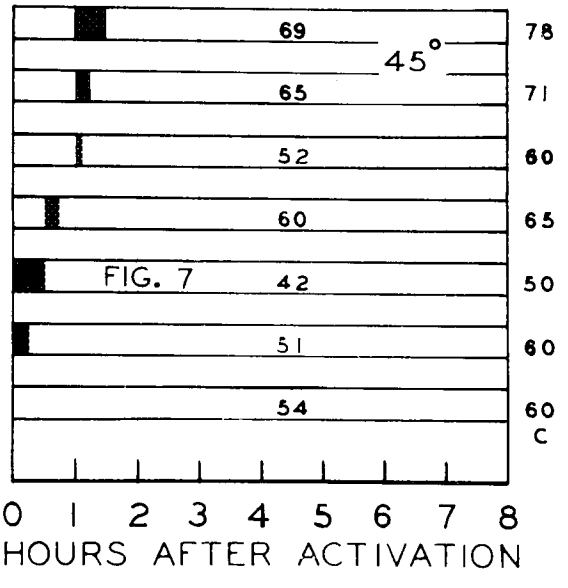
In the experiments on growth, the standardization of inoculum was carried out as mentioned above except that sterile technique was employed. Growth tubes (Ryan, 1950), containing PDA or malt-dextrose agar, were used in these studies. Care was taken to maintain high humidity inside the incubators in which the growth tubes were placed in order to insure that the medium did not dry up during the course of the experiments. Growth was measured every 12 hr. and the average of samples in triplicate was used. Rarely, abnormal growth was observed in a tube, in which case the sample was discarded.

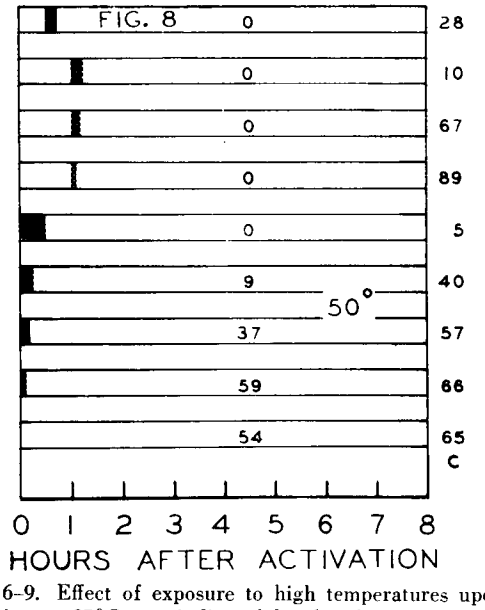
RESULTS. — *Interrupted heat-treatments.* — As stated above, ascospores are normally induced to germinate by a short heat-shock at 60°C., followed by incubation at 27°C. Changes in heat-resistance were studied by means of experiments wherein ascospores were exposed to temperatures between 50 and 70°C. at various times after activation. Controls in all cases were spores activated under the same conditions as the experimental samples and incubated continuously at 27°C. The results of these experiments are provided in fig. 1–4 which show that after 1 hr. of incubation at 27°C., a marked decrease in heat-resistance has occurred in activated ascospores. This is particularly true when the cells are exposed to temperatures greater than 50°C., although there is an effect at this temperature as well. Thus, even 2 hr. at 60°C. reduces germination by only 10%, but such treatment, after the spores have been kept at 27°C. for 1 hr., prevents germination entirely. When incubation at 27°C., after activation, is carried out for 2 hr., treatments as short as 30 min. at 60°C. have a marked effect. It should be noted, in considering the latter data, that 23% of the spores had germinated in the controls at the end of 2 hr. (fig. 1–3), consequently, any heat-treatment administered after this time, would not be expected to affect this background level of germination.

When the temperature to which activated ascospores are exposed is increased, the duration of the treatment required to affect germination adversely is decreased. For example, when incubation at 27°C. for 1 hr. is interrupted by as little as a 5-min. "heat-flash" at 65°C., germination is completely stopped. Yet, exposure to the latter temperature for twice as long, if done immediately following activation, results in only a slight reduction in germination (fig. 3). Similar results were obtained when ascospores were heated at 70°C., although a significant decline in germination occurred as a result of treatment for 15 min., even in the absence of an incubation period at 27°.

The kinetics of the loss in heat-sensitivity was studied by providing a "heat-flash" of 5-min. duration at 65°C. to cells that had been incubated at 27°C. for periods up to 30 min. after activation. After the heat-treatment, the cells were reincubated at 27°C., and the percentage of germination determined as before and plotted in fig. 5. Another set of spores was maintained at 65°C. continuously, for times up to 30 min. after activation, and then placed at 27°C. and their germination determined. Strikingly, even 5-min. incubation at the lower temperature results in the loss of resistance to 65°C. in about ⅔ of the cells. After 25 min. at 27°C., the capacity to resist the high-temperature treatment is lost by all of the ascospores. By con-







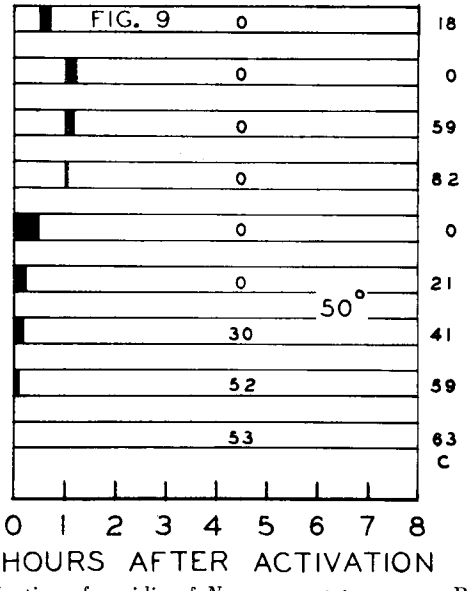


Fig. 6-9. Effect of exposure to high temperatures upon germination of conidia of *Neurospora tetrasperma*. Periods of incubation at 27°C. are indicated by the clear spaces and exposures to higher temperatures by the black. The letter *c* along the ordinate refers to the control which was incubated throughout at 27°C. and the numbers along this axis represent the percentage of germination 8 hr. after the start of incubation. The numbers within the control box are germination percentages at the times indicated below on the abscissa.—Fig. 6. Heat-treatment at 45°C. with strain 394.5. Exposures of 5, 15 and 30 min. were given.—Fig. 7. Heat-treatment at 45°C. with strain 394.4. The same exposure periods as above were used.—Fig. 8. Heat-treatment at 50°C. with strain 394.5. Exposures of 5, 10, 15 and 30 min. were given.—Fig. 9. Heat-treatment at 50°C. with strain 394.4. The same exposure periods as in the experiments outlined in fig. 8 were used.

trast, spores that are incubated continuously at 65°C. after being activated lose their heat-resistance much more gradually. Thus, more than 50% of them germinate after 25 min. exposure to 65°C., followed by incubation at 27°C.

Long periods of exposure to a temperature at which ascospores would be activated (60°C.) were tried. Incubation of dormant cells for as long as 4 hr. at this temperature, followed by 8 hr. at 27°C., resulted in the germination of 30% of the spores. However, germination was never observed to occur while the spores were at the elevated temperature, in spite of the fact that the usual incubation period of 3 hr. at 27°C. suffices to effect complete germination.

Conidial suspensions also were given "heat-

flashes" of different duration at 45 or 50°C., before and after incubation at 27°C., and the percentage of germination calculated. As the results in fig. 6–9 reveal, prior incubation at the lower temperature does not render conidia of either strain of *N. tetrasperma* more sensitive to high temperatures. In fact, when they are treated in this way, the percentage of germination at the end of 8 hr. was usually increased. Thus, in strain 394.5, 30 min. exposure to 45°C., an hour after incubation began, resulted in an increase of 20% in the percentage of germination over that of the control (fig. 6). Similar results were obtained with conidia of strain 394.4, as the data in fig. 7 show. In general, these data paralleled those obtained when conidia were subjected to 50°C., except that germination is delayed beyond 4 hr. in the latter instance (fig. 8, 9). This delay is greater when spores are first incubated at 27°C. than when they are exposed to the higher temperature directly. However, a stimulatory effect upon conidial germination was demonstrable at 50°C., as well as at 45°C., except that the optimal effect was obtained in only 5 min. The inhibitory effect of such treatment is greatest when conidia are subjected to high temperatures prior to incubation at 27°C. Thus, the germination of strain 394.4 is completely stopped by 30 min. treatment at 50°C., while those of strain 394.5 are influenced almost as much. The same effect is apparent at 45°C. but is less marked. Parallel experiments carried out with 5-min. "heat-flashes" at 60°C. resulted in the complete prevention of germination, no matter when the treatment was administered.

Cardinal temperatures for growth and germination.—Ascospores were activated in suspension, as described previously, and then were spread upon the surface of PDA in Petri dishes and incubated at various temperatures. Germination was fastest and most complete at 27°C., although more than ½ of the spores germinated at 44°C. (table 1). On the other hand, no germination occurred at temperatures above 44°C. The limit for the germination of ascospores is about 5°C. since only a few spores germinated at this temperature after 3 days.

TABLE 1. *Germination of ascospores of Neurospora tetrasperma at different temperatures; dashes indicate no counts were made*

Time of incubation (hr.)	Percentage germination at:				
	5°C.	9°C.	27°C.	44°C.	45°C.
2	0	0	23	0	0
4	0	0	89	0	0
6	0	0	92	45	0
8	0	0	96	53	0[a]
24	0	11	—	—	0[a]
48	0	23	—	—	0[a]
72	8	—	—	—	0[a]

[a] A few germ buds appeared but did not progress to form a germ tube.

The temperature-maxima for the growth of the 2 strains of *N. tetrasperma*, as well as for 2 of *N. crassa*, were also studied. Inocula from the several sources were prepared and incubated at different temperatures in growth tubes on PDA. None of the strains of *N. tetrasperma* used grew above 40°C. and growth was greatly restricted at this temperature (table 2). This was so despite the excellent germination of conidia of strain 394.5 even at 44°C., so that growth itself was affected and not simply germination. The same is true, to a lesser extent, for strain 394.4, inasmuch as 10% germination was noted at 44°C., and for mycelium produced from ascospores in which 53% germination occurred. The most resistant of the strains tested was 4A of *N. crassa*, which grew slowly at 45°C., whereas strain 12a has a maximum temperature for growth comparable to that for *N. tetrasperma*.

TABLE 2. *Growth of Neurospora at different temperatures. Readings represent averages of the growth in mm. of triplicate samples during a 24-hr. period; the figures in parentheses are the germination percentages determined from parallel cultures on PDA in Petri plates*

Source of inoculum	Growth in mm. at:				
	35°C.	40°C.	44°C.	45°C.	46°C.
N. tetrasperma:					
ascospores	104	17	0 (53%)	0 (0)	0 (0)
conidia (strain 394.4)	86	0 (17%)	0 (10%)	0 (0)	0 (0)
conidia (strain 394.5)	87	12 (80%)	0 (77%)	0 (8%)	0 (0)
conidia (strains 394.4 × 394.5)	92	12	0	0	0
N. crassa:					
conidia (strain 4A)	85	23	—	11	—
conidia (strain 12a)	108	10	—	0	—

Effect of removal of exospore upon heat-resistance and activability.—The work of Lowry and Sussman (1958) has disclosed that the wall of ascospores of *N. tetrasperma* consists of 3 principal layers. The outermost one, or exospore, is characterized by its longitudinal striations which comprise the "nerves" which give the organism its name. This wall layer can be removed, by the use of Clorox, with no apparent effect upon the viability of the cell or upon the structure of the parts which remain after such treatment. Consequently, it was possible to study the effect of the removal of the exospore upon the heat-resistance and response to activation of ascospores.

Heat-resistance was studied first by subjecting "uncoated" ascospores to "heat-flashes" of the kind used above for the untreated cells. However, when such spores were heat-activated at the usual tem-

perature (60°C.), very little germination was obtained. This suggested, therefore, that the optimal conditions for activating "uncoated" spores had to be defined. Therefore, they were exposed to several temperatures for varying periods and the percentage of germination was determined. As the results in table 3 disclose, the optimal temperature for

TABLE 3. *Effect of wet and dry heat upon the dormancy of ascospores of N. tetrasperma, from which the exospore had been removed; after heat-treatment all samples were incubated at 27°C. for 4-5 hr. in water. Dashes indicate that no readings were made*

	Percentage germination			
	Duration of heat-treatment (min.):			
Temperature	5	10	15	20
Wet-heat:				
60°C.	10	14	15	7
50°C	50	60	53	30
45°C.	30	34	49	33
40°C.	26	32	34	40
35°C.	26	30	32	35
30°C.	23	26	29	34
27°C.			21 [a]	
21°C.			12 [b]	
Dry-heat:				
60°C.	11	14	—	11
50°C.	25	15	—	11
45°C.	39	47	—	34
40°C.	27	27	—	22
35°C.	24	27	—	18

[a] Ascospores were continuously incubated at 27°C.
[b] Ascospores were continuously incubated at 21°C.

activation of "uncoated" ascospores is about 50°C. Moreover, significant activation is accomplished even at 30°C., and some spores germinated with no treatment other than incubation at 27°C. or 21°C., so that the requirement for heat-activation has been considerably mitigated by the removal of the exospore. This was shown in another way by the use of dry heat instead of wet as in the previous experiments. The data in table 3 show that "uncoated" ascospores are activated optimally at 45°C. by dry heat, although the total amount of germination is lower than when wet heat is used.

In contrast, the untreated ascospores were activated to a maximum of only 17% when dry heat at 60°C. was used (table 4). That the spores were not killed by this treatment was shown by the fact that subsequent exposure to 60°C., in water, resulted in activation to a degree almost equivalent to that in controls exposed only to wet heat. It should be pointed out that dry heat at 50°C. or 40°C. was totally ineffective in breaking the dormancy of these cells.

DISCUSSION AND CONCLUSIONS.—To the list of changes which occur upon the disruption of the dormancy of ascospores of *Neurospora* can now

TABLE 4. *Effect of wet and dry heat at 60°C. upon the dormancy of ascospores of N. tetrasperma; the spores were suspended in dist. water after the heat-treatment and incubated at 27°C.*

Time of heating (min.)	Percentage germination	
	Dry-heat	Wet-heat
5	1	73
10	2	85
15	3	87
20	7	79
25	10	76
30	17	76
45	6	75

be added loss in heat-resistance. Evidence of this change is found within 5 min. after activation, and the loss of such resistance is almost complete within ½ hr. thereafter, that is, at least 90 min. before any morphological change is perceptible. Some hint as to the site of the ascospore's heat-resistance is furnished by the experiments with "uncoated" spores. The diminished resistance of such cells to high temperatures (table 3) and their activation even at temperatures as low as 30°C., suggest that the exospore, or an associated structure, is removed or altered by Clorox and is responsible for the thermal-tolerance of ascospores. These observations recall the fact mentioned by Faull (1930) and Dodge (1930) that asci from certain crosses of *Neurospora* yielded ascospores that germinated without being heat-activated. It would be pertinent to determine whether there is any difference between the wall of these spores and that of cells requiring heat-treatment.

Previous work has shown that although activation occurs at 60°C., the subsequent steps which culminate in germination do not proceed unless the spores are incubated at lower temperatures (Lingappa and Sussman, 1959). Moreover, these workers showed that the utilization of the ascospore's pool of soluble carbohydrates was slower at the higher temperature than at 27°C. These data are complemented by the results of the experiments reported herein which show that germination never occurs if spores are kept at 60°C., even though viability is retained. Inasmuch as loss in heat-sensitivity has been shown to be a concomitant of activation, the fact that short periods of incubation at 27°C. are required before such a change occurs is further evidence for the necessity of incubation at moderate temperatures.

A requirement for incubation at a temperature lower than that which breaks dormancy appears to be general among organisms which require such treatment. This is true for urediospores of *Phragmidium mucronatum* (Cochrane, 1945), spores of *Ustilago striiformis* (Kreitlow, 1943), as well as for several other organisms listed by Sussman (1959).

The several stages in the life-cycle of *Neurospora* have temperature-maxima that differ markedly. Ascospores, of course, are the most resistant, for not only do they survive 4 hr. at 60°C. but they are activated, if an incubation period at 27°C. is not interposed. These observations confirm the heat-resistance of ascospores which was studied in detail by Faull (1930), who reported that they survived heating for more than 4 hr. at 50°C. Conidia, on the other hand, are killed by 5-min. exposure to 60°C., so that they are much more susceptible to injury by high-temperatures than are ascospores. By contrast, Faull showed that conidia survived exposures to −170°C. when dry almost as well as did ascospores. Judging from the temperature-maximum for the growth of the mycelium of *N. tetrasperma,* the vegetative phase in the life-history is the least resistant to high temperatures. Thus, in this case, none of the strains tested grew at temperatures above 40°C., even though both ascospores and conidia germinated in high percentage at 44°C. Despite the difference in heat-resistance of ascospores and conidia discussed above, their temperature-maxima for germination are about equivalent. In fact, those of conidia of strain 394.5 of *N. tetrasperma* and of strain 4A of *N. crassa* are higher than that for ascospores. The explanation of this seeming paradox lies in the change in heat-resistance of ascospores within a short time after germination, as discussed above. That strain- and specific differences in heat-resistance may occur is also pointed up by these data, inasmuch as strain 394.5 of *N. tetrasperma* is more resistant than 394.4, and strain 4A of *N. crassa* is the most resistant of all those tested. On the other hand, there is no interaction between the strains of *N. tetrasperma* when they are crossed, in that the rate of growth of the mycelium derived from this cross is equivalent to that of the most resistant of the two.

The lesser effect of dry heat, as compared with that of wet, upon the time required for germination, has been observed previously in *N. crassa* (Faull, 1930). Similarly, Tsaugi (1933) found that oospores of *Sclerospora graminicola* remained viable at 60°C. when dry, but that they were killed in water. Furthermore, as was mentioned above, Faull also showed that ascospores and conidia subjected to −170 to −190°C. survived longer when dry than wet, thereby extending the conclusion discussed above to include extreme cold. The present results are in agreement in that only slight activation of ascospores is induced by dry-heat. On the other hand, there are reports that germination is stimulated in *Phragmidium mucronatum* under these conditions (Cochrane, 1945), so that the conclusion cannot be generalized.

A similarity between ascospores and conidia involves their increased germination after heat-treatment. The response of ascospores to such treatment is well known but that of conidia was called attention to only recently by Ishii and Miyamota (1954). These workers reported that exposure of conidia of *N. sitophila* to 35–37°C. for 1–3 hr. induced increased germination. The results in fig. 6–9 corroborate these findings and disclose that 5 min. at 50°C. or 30 min. at 45°C. have a stimulatory effect. However, these experiments, as well as those of Ishii and Miyamoto, reveal that the effect of heat upon the germination of conidia is not absolute in that some occurs in the absence of such treatment.

The results with conidia are at variance with those obtained by Mitchell (1957) who exposed conidia of strain C–102 of *Neurospora crassa* to 60°C. for 30, 60 and 120 sec., at various times after the start of germination. Thus, when conidia were treated at 60°C. for 30 sec., the percentage of survivors dropped from 100% to 2% after 2 hr. of incubation. These results are analogous, therefore, to the ones obtained with ascospores in the present work, but a direct comparison with the data on conidia is not possible because no heat-treatments at 60°C. shorter than 5-min. duration were administered.

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SEGREGATION AND RECOMBINATION OF CHEMICAL CONSTITUENTS IN A HYBRID SWARM OF BAPTISIA LAEVICAULIS × B. VIRIDIS AND THEIR TAXONOMIC IMPLICATIONS [1]

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ABSTRACT

TURNER, B. L. (U. Texas, Austin), and RALPH ALSTON. Segregation and recombination of chemical constituents in a hybrid swarm of Baptisia laevicaulis × B. viridis and their taxonomic implications. Amer. Jour. Bot. 46(9): 678–686. Illus. 1959.—Selected plants from a hybrid population of *Baptisia laevicaulis* × *B. viridis* and individuals from "pure" populations of the parental species were examined chromatographically. The resulting patterns were then compared with the respective phenotypes by the use of hybrid indices. Although the parental type individuals yielded nearly uniform patterns, hybrid-type plants showed a striking recombination of chemical components and there was an excellent correlation between chromatographic patterns and hybrid expression. Of particular interest was the detection of a new and distinctive compound in 2 of the hybrid-type plants which was not observed in either parent. Chromatograms of 3 other *Baptisia* species also showed distinctive patterns, and their relationship to each other, as indicated by morphological features, was accompanied by similarities in the biochemical pattern. The segregation and recombination of biochemical constituents are discussed with respect to their evolutionary and taxonomic implications. It is suggested that an extension of the techniques utilized might provide for the establishment of "biochemical profiles" which should prove of considerable value to the systematist.

THE GENUS *Baptisia* (family Leguminosae) contains about 30 species widely distributed in the eastern United States. Larisey (1940a) has been the only recent monographer of the group, and in her treatment she has recognized a number of intraspecific taxa as well as several named hybrids. That hybridization is common between the various taxa may be inferred from the fact that Larisey recognized numerous cases of natural hybridization involving at least 8 different species.

In southeastern Texas, 4 well-marked species (*B. viridis*, *B. laevicaulis*, *B. leucantha*, and *B. nuttalliana*) are frequently found growing together in various combinations and, in such cases, especially in disturbed habitats, hybridization is characteristic. A detailed morphological study of a hybrid swarm involving *B. viridis* and *B. leucantha* was reported by Larisey (1940b), and Turner (unpublished) has made a similar morphological study of hybrid swarms involving *B. viridis* and *B. laevicaulis*. Hybrid swarms between the latter species are particularly common and occur in varying degrees

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