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Comparative Morphology and Respiration of Normal and Diseased Helminthosporium Victoriae.

Evangelos Eleftheriou Psarros
Louisiana State University and Agricultural & Mechanical College

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Louisiana State University, Ph.D., 1961
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COMPARATIVE MORPHOLOGY AND RESPIRATION OF NORMAL AND DISEASED HELMINTHOSPORIUM VICTORIAE

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment for the degree of Doctor of Philosophy in

The Department of Botany, Bacteriology and Plant Pathology

by

Evangelos E. Psarros
Diploma, Agricultural College of Athens, Greece, 1954
M.S., Louisiana State University, 1959
January, 1961
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ABSTRACT

A comparative morphological cytological study of normal and diseased cultures of *Helminthosporium victoriae* revealed certain irregularities that characterized the diseased colonies. Young hyphae from newly diseased colonies showed various degrees of lytic degeneration and attempts to detect nuclei in such hyphae were unsuccessful. Similar to normal *H. victoriae*, young hyphae from diseased colonies obtained by subculturing from severely diseased mycelium did not show any signs of lytic degeneration and contained abundant nuclei. These hyphae did, however, show various kinds of distortions in the morphology of cells and in the pattern of growth.

In comparative respiratory studies, diseased *H. victoriae* from 10-day old stationary and from 2- to 6-day old shake-cultures showed an increased respiratory rate compared with that of the normal fungus. In addition, diseased *H. victoriae* from 4-day old shake-cultures did not show any increase in respiratory rate in the presence of low concentrations of DNP (5×10⁻⁵ and 1×10⁻⁴ M), whereas the normal fungus showed a positive response.

Comparative study of the process of infection of susceptible oat seedlings with normal and diseased *H. victoriae* revealed little differences. However, the degeneration of tissue inoculated with the diseased fungus was slower and less extensive than that inoculated with the normal fungus.
INTRODUCTION

Victoria blight of oats caused by Helminthosporium victoriae Meehan and Murphy was first observed in the field in 1945 (43). In 1946, it had become a major disease of oats in several states. Oat varieties possessing the "Victoria type" of crown-rust resistance were highly susceptible to Victoria blight, while varieties lacking this type of rust resistance were not susceptible (28, 40, 47). In the following year, the disease spread throughout the oat growing areas of the United States wherever Victoria hybrids were grown. It was also found in Louisiana (58). The fungus invaded the host tissue to only a limited extent, but severely damaged and even killed the susceptible oats by the production of a highly specific toxin (victorin) (42, 44, 50, 66, 67).

In recent years, there have been fewer epiphytotics of this disease in the South, even though considerable acreages of oats with Victoria parentage have been planted. Among isolates from blighted Victor-grain oats Lindberg (36) observed that several colonies of H. victoriae were abnormally stunted. It was found that the abnormally stunted colonies contained some transmissible agent capable of producing the abnormality in normal colonies. Tests for the presence of bacteria or a toxin that might be responsible for the abnormality of the colonies gave negative results. On the other hand, he could find no
activity in filtrates of clarified abnormal juice after passage through bacteriological filters. More recent investigations with cell free filtrates have also failed to demonstrate a causal agent (39). Lindberg concluded that the causal agent was either not present in the filtrates or not capable of infecting normal *H. victoriae* independent of viable fragments of the diseased fungus. A comparison of normal and diseased *H. victoriae* has revealed marked differences in pathogenicity and toxin production but has not provided a definitive identification of the causal agent (37, 38).

The present study is an attempt to make further basic comparisons between normal and diseased *H. victoriae* by studying differences in the morphology and in certain metabolic activities, such as the respiratory rate and the respiratory response to 2,4-dinitrophenol. Further, by studying comparatively the phenomenon of infection in artificial inoculations of susceptible oats with normal and diseased *H. victoriae*, an attempt was made to obtain some information concerning the behavior of the diseased fungus, which might account, apart from the observed less toxin production, for the significantly lower pathogenicity exerted by this fungus.
The great variability existing within single species of fungi has been recognized for a long time. According to Ingold (25), Albrech von Haller more than two hundred years ago, described fungi as a "mutable and treacherous tribe." Major contributions to the study of variability in species of *Helminthosporium*, in particular, are the early works of Stevens (60), Christensen (8), and Christensen and Graham (9). Working with *Helminthosporium gramineum*, the last two authors came to the conclusion that this species comprised an indefinite number of "races" which differed from one another in many morphological and physiological characteristics including pathogenicity. Christensen, Stakman and Christensen (7), and more recently Buxton (6) and Johnson (27) have reviewed the subject of variation in plant pathogenic fungi. In general, variation other than that due to nutritional or other environmental factors has been interpreted by various genetic mechanisms, such as mutation, hybridization, heterokaryosis, and parasexual recombination. There are, however, in the literature many cases of variation characterized as saltation (often called dissociation or sectoring of fungus colonies), or attenuation (often called dissociation leading to loss of virulence of the pathogen), or adaptation (tolerance to poisons, adaptation in pathogenicity), the mechanism of which is not yet well understood (6).
Among the various types of variants, which have been studied intensively from a genetical and metabolic point of view, are a group of variants with markedly restricted growth on any medium on which they have been planted. Such variants derived spontaneously or after irradiation have been noticed in the ascomycete *Neurospora crassa* and in the baker's yeast, *Saccharomyces cerevisiae*. Mitchell and Mitchell (46), Haskins *et al* (21), and Tissieres *et al* (62) have studied the slowly growing strain, designated as "poky" of *Neurospora*. They concluded that the poky characteristics were inherited through the cytoplasm, since they were transmitted to offspring only through the "maternal" or protoperithecial parent (46). The presence of a virus or other infectious agent in poky was doubtful because mixed wild type and poky cultures failed to effect the growth of the wild type strain (46). Mitchell and Mitchell (46) stated that there was no evidence that heterokaryons had been produced from the mixture of poky and wild type *Neurospora crassa*. Gowbridge (17), on the other hand, made heterokaryons between strains of *Neurospora crassa* having poky and wild type cytoplasms. Poky was not dominant over the wild type in the way which might be expected if poky condition were caused by an infective agent. It was shown by biochemical studies that the slow growth of poky was due to a respiratory deficiency (the $Q_2$ of poky was roughly one-third that of the wild strain), which, in turn, was the result of a deficiency in cytochrome a and b and thus in cytochrome oxidase and succinic acid oxidase (21, 62). Later, Tissieres and Mitchell (61) reported the finding of
three other strains in *Neurospora* which, like poky, had deficiencies in cytochrome systems and lower respiration rates. Similarly, Ephrussi (14) has attributed the slow growth of "vegetative little" colonies or "petites" in baker's yeast to a low respiration which was due to cytochrome deficiencies. The petite characteristics were retained indefinitely in the course of vegetative reproduction. Ycas and Starr (72) and Harris (20) have also found dwarf strains of yeast characterized by deficiency in enzyme systems and low respiration. Ycas and Starr (72) stated that their dwarf strain showed no respiratory activity. According to Cochrane (11), correlation between inhibition of growth and inhibition of respiration was usually found in fungi affected by antifungal chemical agents. Although metabolic studies of pathological abnormalities in fungi have been insufficient to justify any generalization, the foregoing reports seem to suggest that reduced respiration parallels reduced growth of fungi whether due to genetic or fungicidal factors.

In contrast, in the field of plant pathology a considerable amount of work has been done in studying metabolic alterations in diseased plants. In recent years, in particular, more and more workers have tried to determine the mechanisms controlling metabolic activities in diseased plant tissues and thus elucidate the underlying principles of the nature of disease. Several articles, such as those by Allen (2, 3, 4), Kern (30), Parkas and Kiraly (16), Kirali and Parkas (32), and Uritani and Akazawa (64) are helpful in understanding this problem. The general
idea is that the biochemical alterations of a diseased plant are actually the "primary" symptoms of its disease, and that the nature of the metabolic response may be more characteristic of the disease concerned than the visible "secondary" symptoms.

According to Uritani and Akazawa (64), among the many metabolic changes observed in diseased plant tissues, alteration of respiration is one of the most fascinating subjects, since the respiratory mechanism occupies a central part of metabolism. An increase in the respiratory rate has been reported as a typical feature of the metabolism of plant tissue following infection either by a fungus, a bacterium or a virus. When a disease has led to a degenerated condition of the plant tissue the respiratory rate may decline and eventually stop. The above generalization has been derived from review of a large number of works. Wynd (69), Allen (2), and Uritani and Akazawa (64) have included in their reviews more than fifty different reports of an enhanced respiration in diseased plant tissues. Different plant material, such as sweet potato roots, Irish potato tubers and leaves, lemon fruits, tomato, tobacco, cucumber, cabbage, spinach, sugar beet, wheat, barley, rice, clover, bean, raspberry, peach, aster, Nicotiana glutinosa, Euphorbia peplus and Althea rosa leaves, safflower hypocotyls, and cotton stems were studied. Several fungi with different degrees of parasitism and several bacteria and viruses were involved as pathogenic agents. More recent reports on the same subject, such as those by Wolf and Schramm
(68) with tobacco infected with black shank, Loebenstein (41) with sweet potato leaves infected with vein-clearing virus, and Yamaguchi (70) with the plant-virus combinations *Datura stramonium* - tobacco mosaic virus, *Vicia faba* - cucumber mosaic virus, and *Gomphrena globosa* - potato virus X, are in confirmation with the above consideration. Grimm (18), working with the Victoria blight of oats, also found similar results. Several of the reported experiments have shown that the enhancement of the respiratory rate of the infected plant tissues should be attributed, at least in part, to an independent phenomenon exhibited by the plant tissue rather than being due solely to the respiration of the invading microorganism.

Several explanations have been proposed for the enhanced respiratory rate of diseased plant tissue. One of them is the possible "uncoupling" of oxidative phosphorylation from respiration, so that respiration would no longer be limited by insufficient concentrations of ADP and inorganic phosphate. It has been proposed that uncoupling could be caused by a toxin produced by the pathogen (2, 45), an abnormal metabolite of the diseased tissue (64), the destruction of mitochondria or the operation of an alternative respiratory pathway other than the TCA cycle-cytochrome system (15, 35, 56, 64). Another explanation proposed for the mechanism of respiratory increase is the acceleration of ATP-utilizing reactions in the diseased tissue (3, 4, 64). Uritani and Akazawa (64) have listed such processes as accumulation, mobilization,
and synthesis of phosphorus and carbon compounds, growth of the host tissue, synthesis of protein including activation of enzymes and enzyme systems, and increase in protoplasmic activity. Another proposed explanation is the abolition of the Pasteur effect in metabolism of diseased plant tissue, and, consequently, the suppression of carbohydrate breakdown under aerobic conditions as compared to that under anaerobic conditions (2, 12, 15, 16, 53, 56, 64). Several hypotheses have been proposed to interpret the Pasteur effect, such as the relative shortage of ADP and inorganic phosphate during effective operation of oxidative phosphorylation, and the presence of some unstable system in the oxidized mitochondria that may inhibit hexokinase or phosphofructokinase (64). Another explanation proposed for the increased respiratory rate is that some alteration in concentration or activation of respiratory enzymes, with particular reference to terminal oxidase, may influence the overall respiration mechanism in diseased plant tissue (16, 31, 34, 54, 65, 71).

From the foregoing considerations it is suggested that there are not yet sufficient data to support any of the proposed mechanisms as the predominant one accounting for the respiratory increase in diseased plant tissue. This seems to justify the assumption that a possible combination of more than one of these or other yet unpostulated mechanisms may also operate instead of a single factor, and, further, that the combination may differ with particular diseases. To throw some light on this obscure problem, students of metabolic alterations in diseased
organisms have used certain substances which interfered with the metabolism and produced certain characteristic responses. One of the most extensively studied compounds of this sort is 2,4-dinitrophenol (DNP). Low concentrations of this compound cause marked increases in the respiration of higher plant tissues and of many microorganisms, while higher concentrations of it inhibit respiration and eventually suppress it (5, 10, 19, 24, 29, 48, 49, 52, 57, 59). Although the exact site or sites of action of DNP are not yet definitely known, low concentrations are considered to cause an uncoupling of oxidative phosphorylation from respiration, and thus, to permit respiration to go at a higher rate. Diseased plant tissues are considered to be less sensitive to the same low concentrations of DNP as compared with healthy tissues. Accordingly, Akazawa and Uritani (1) have found that two to four days after inoculation of sweet potato roots with Ceratostomella fimbriata, when the respiratory increase showed a maximum value, the percentage increase of respiration due to DNP addition was considerably smaller than in healthy control tissue. According to Uritani and Akazawa (64), Kiraly and Farkas, using wheat seedlings infected with rust, also observed a decreased respiratory rate in the DNP treated tissues in comparison with a positive DNP response in the healthy tissue. Shaw and Samborski (56), working also with rusted wheat leaves, have found a decreased percentage response to DNP. Similarly, Grimm (18) working with oats infected with Helminthosporium victoriae found that during a course of eight days after inoculation the respiratory increase due to
DNP was always lower than in control healthy tissue. When the respiratory peak was reached in the control tissue 4 days after inoculation, the response of the infected tissue to DNP was almost nil. According to Parkas and Kiraly (16), the reduced respiratory response to DNP in diseased tissue may be explained on the assumption that DNP cannot exert any uncoupling action in this tissue, since the ATP/ADP ratio has already been reduced as a result of the disease.

The foregoing review seems to justify the conclusion that the following two features may be considered characteristic of the disturbed metabolism of infected plant tissue: (1) an enhanced respiratory rate, and (2) a reduced respiratory response to low concentrations of DNP.
MATERIALS AND METHODS

Fungus cultures

 Cultures of normal and diseased *Helminthosporium victoriae* of isolate 1, designated by Lindberg (38) as N-1 and D-1 respectively, were the origin of all cultures used in this study. A spore suspension was prepared aseptically from the normal culture, poured into plates containing 2 per cent agar and kept open for a short time to allow the water to evaporate. Single conidia were picked with a pointed inoculating needle and transferred to agar plates. Hyphal tips from the germinating conidia were isolated to fresh culture media. One such single-spore isolate designated SN-1 was used throughout these studies as the stock culture for the normal fungus and propagated by subculturing on PDA. Constant care was taken during the propagation to avoid any sectoring or other macroscopical deviation from the original isolate. The disease or abnormality was transmitted to normal SN-1 by the method used by Lindberg (36) and the diseased culture has herein been designated as SD-1. It was also propagated by subculturing on PDA.

Morphological studies

 A suitable method for preparing material for microscopic examination without disturbing the growth habit and other features of mycelium was mostly used (51). Cellophane pieces about 3 x 1 cm were placed in distilled water and sterilized by boiling or autoclaving for 15 minutes.
The cellophane pieces were transferred aseptically to the surface of PDA culture plates and arranged radially. Inoculum from normal or diseased fungus placed in the center of the culture plates grew on the upper surface rather than between the cellophane and agar. The cellophane fungus preparations were carefully removed from the plates and placed on microscope slides for examination either directly or after fixing and staining. The following three staining methods were used.

Iron-propiono-carmine was prepared by dissolving 1 g of carmine in 100 ml of boiling 45 per cent propionic acid and incorporating the iron by dipping a dissecting needle in the still warm dye. One or two drops of the combined stain and fixative were placed on the cellophane fungus preparation on a microscope slide and a coverslip added. In some instances the preparation was heated gently for a few minutes before the coverslip was sealed with melted paraffin (55).

A modified hematoxylin staining mountant (26) was prepared by dissolving 10 g of finely ground gum arabic with gentle heating in a solution of 5 ml formic acid in 25 ml distilled water. Two-tenths gram of hematoxylin, 0.8 g of ferric alum and 0.2 g of chrome alum were ground to a fine powder and dissolved in the gum arabic solution. The mixture was then placed in an embedding oven at 60°C for 24 hours. After the preparation had cooled to room temperature, it was centrifuged at 20,000 x gravity for 45 minutes. After decanting the preparation from the centrifuge tubes, 12 ml of glycerine and 12 g of chloral hydrate were incorporated by gentle stirring. Only a few drops of the staining mountant
preparation were needed for each slide.

The giemsa staining method (22, 23, 33) was also used. The cellophane fungus preparations were left in a killing and fixing mixture of 3 parts of absolute ethyl alcohol and 1 part of glacial acetic acid overnight, transferred to 95 per cent alcohol for 10 minutes, and stored in 70 per cent alcohol. The fixed material was placed in distilled water for 10 minutes, transferred to cold 1N HCl for 10 minutes, immersed in 1N HCl at 60°C for 10-12 minutes, and then stained for 15 to 30 minutes or more. To make the staining solution, 0.5 g of giemsa powder (National Aniline Division, cert. No. NGe 17) was dissolved in 33 ml glycerin at 55°C-60°C for 1 1/2 to 2 hours. To this 33 ml of methyl alcohol was added. One or two drops per ml of this liquid were added to a phosphate buffer solution. The buffer solution contained 50 ml of M/5 KH₂PO₄, enough M/5 NaOH to bring the pH up to 6.9, and distilled water to make a final buffer solution of 200 ml. Finally, the cellophane fungus preparations were removed from the staining solution, rinsed several times in distilled water and once in buffer, and placed on microscope slides. Semipermanent mounts were made in buffer solution saturated with Abopon.

Respiration studies

Five- to 10-day old subcultures of normal (SN-1) and diseased (SD-1) H. victoriae were used as a source of inoculum for all respiration studies.
Preliminary experiments were carried out by using mycelium from stationary cultures in a modified Fries' liquid medium (42). Fifty ml of medium in 250-ml Erlenmeyer flasks was inoculated with a 3-mm cube of mycelial mat plus agar. The flasks were incubated at $26^\circ + 0.5$ C for 10 days, the mycelial mats washed with distilled water, cut into 2-3 mm pieces, and washed several times with distilled water and 0.05 phosphate buffer of pH 5.0 in a Buchner funnel. About 300 mg wet mycelial pieces were placed in the main compartment of each respiration vessel of a Warburg manometric apparatus together with 2 ml of the buffer. One half ml of 0.1 M sucrose solution was added in the side arm. Readings were recorded at 15-minute intervals after a 15-minute equilibration period. The dry weight and nitrogen content were determined at the end of each experiment. The nitrogen content determinations were made by a micro-Kjeldahl procedure.

Later experiments were carried out by using mycelial pellets from shake cultures in 250-ml Erlenmeyer flasks. Fifty ml of the above mentioned Fries' medium were added to each of several replicate flasks. Flasks were inoculated with a mycelial suspension prepared by macerating mycelial mat covering approximately half the surface of a petri plate in 100 ml of sterile water in a Waring blender for 1 minute. Two ml of this suspension were added to each flask. The flasks were then placed in a tilting position on a shaker having 180 circular movements a minute at $24^\circ$ to $25^\circ$ C.
At the end of the incubation period, the pellets were removed from the flasks, washed several times with distilled water in a Buchner funnel or a coarse sintered glass funnel, and washed once with 0.05 M phosphate buffer at pH 5.0. Wet pellets were coarsely fragmented in a Waring blender for 5 seconds in 5 parts of buffer. One and one-half ml of the suspension was pipetted in the main compartment of each respiration vessel, to which 0.5 ml of buffer alone or the desired concentration of DNP was added. One-half ml of 0.05 M solution of anhydrous dextrose was pipetted in the side arm. A folded filter paper crimp weighing 0.03 g and 0.2 ml of 20 per cent KOH were added to the center well. Carbon dioxide production was measured with parallel vessels from which potassium hydroxide was omitted. All experiments were carried out at 25°C with air as the gas phase. Readings were recorded at 30-minute intervals after a 30-minute equilibration period. Two, three, or four replicate vessels were used for each treatment. The dry weight determinations were made at the start on aliquots of the mycelial suspension, which were allowed to dry for three days in an oven at about 80°C. The nitrogen content was determined on the same aliquots by a micro-Kjeldhal procedure.

**Infection studies**

Oat seedlings were grown in the greenhouse in 8-inch pots and inoculated in the 2-3 leaf stage. A 3-mm cube of mycelial mat plus agar was placed in the axile of the primary leaf with the fungus portion
in contact with the leaf blade. The inoculum was covered with a small piece of moistened absorbent cotton. The inoculated pots were placed in a moist chamber or left on a bench and covered with glass bell jars. Pieces of inoculated leaves 1/2 to 1 cm long were taken 6, 9, 12, 18, 24, 36, 48, 72, 96, and 120 hours after inoculation, and killed and fixed in an acetic acid-alcohol fixative (mixture of equal parts of 95 per cent alcohol and glacial acetic acid). After two days, the leaf pieces were cleared in 70 to 75 per cent lactic acid for several days at room temperature (13). The cleared material was taken from the clearing agent and the excess lactic acid removed with blotting paper. Whole mounts and free-hand sections were stained 10 to 20 minutes with acid fuchsin in lactophenol (5 ml of 1 per cent aqueous solution of acid fuchsin and 100 ml of Aman's lactophenol). The excess stain was removed with blotting paper and the stained material was rinsed and mounted in lactophenol. Free-hand sections were also examined directly, without cleaning and staining.

Paraffin sections often showed some collapsing of the tissue, and no staining procedure was found which gave satisfactory differentiation between the mycelium and the host tissue.
EXPERIMENTAL RESULTS

Morphological study of normal and diseased H. victoriae

Normal H. victoriae transplanted to culture plates containing cellophane pieces grew rapidly at room temperatures. In 4-5 days the mycelium covered the surface of the plate with a dense, tufted colony. The color of the colony was light greenish during the period of rapid growth, but later turned to medium gray. It has been shown that normal H. victoriae dipped into a fragmented suspension of diseased fungus gave rise to colonies with well established symptoms of the disease within 72 hours (36). The inoculated colonies developed diseased sectors where growth of the fungus almost completely stopped.

The disease spread through the young hyphae at the margins of the colonies and much of the aerial mycelium behind the margins collapsed. During the present study, inoculum was taken from diseased areas of newly inoculated colonies and propagated by successive transplantings. During this propagation, constant care was taken to use inoculum from severely diseased mycelium. Such subcultures resulted in very small, brownish-gray, diseased colonies with irregular shapes. Submerged mycelium was very dense and often extended to the lower surface of the agar, while the aerial portions were scanty and often completely absent at the margin. At other times, submerged mycelium grew in isolated areas from which scanty aerial tufts emerged.
Young hyphae growing on cellophane pieces from normal colonies, newly inoculated colonies, and small colonies derived from successive subcultures of diseased colonies, were stained and examined microscopically. Those from normal colonies were long, mostly straight, with a few lateral branches, and often arranged in bundles (Figs. 1, 2, 3). The width of the older portions of the hyphae measured 4 to 6 u, gradually tapered to 1.5 to 3.5 u at the tips, and bulged slightly at the septations. The length of cells ranged from about 15 u at older portions of hyphae to 50 u or more at younger portions. In preparations stained with giemsa, several nuclei (mostly 4-6) appeared in each cell (Fig. 4).

Young hyphae from newly diseased colonies generally grew singly and were aligned in different directions. The dimensions of the hyphae did not differ appreciably from those of the normal fungus, but the young hyphae of newly infected colonies showed various degrees of lysis and a variety of distortions (Figs. 5, 6, 7, 8). The apical portions were sometimes twisted and swollen, and their lateral branches were short and slightly swollen. Many cells showed a degeneration or lysis of the protoplasm. Unlike the normal fungus which had a more or less transparent and uniformly stained cytoplasm throughout the cell, the cytoplasm of the degenerating cells appeared to contain granules of different shapes and sizes, and stained slightly differently. Sometimes, only portions of such cells had lost their protoplasm, while in other
Figure 1. Normal *H. victoriae* stained with iron-propiono-carmine.

Figure 2. Normal *H. victoriae* stained with hematoxylin.
Figure 3. Normal *H. victoriae* stained with giemsa.

Figure 4. Normal *H. victoriae* stained with giemsa and showing nuclei.
Figure 5. Newly diseased *H. victoriae* stained with iron-propiono-carmine.

Figure 6. Newly diseased *H. victoriae* stained with iron-propiono-carmine.
Figure 7. Newly diseased *H. victoriae* stained with hematoxylin.

Figure 8. Newly diseased *H. victoriae* stained with giemsa.
cases the cells appeared to be completely devoid of protoplasm. In many such cases globular masses of protoplasm were exuded outside the cell, and were closely appressed on the cell wall. In degenerating portions of the hyphae attempts to detect nuclei were unsuccessful.

Cellophane preparations from diseased colonies derived from successive subcultures of diseased *H. victoriae* were covered with densely packed hyphae (Figs. 9, 10, 11). Such hyphae grew in different directions and were short and greatly branched. Their widths varied greatly from cell to cell and even within single cells, measuring from 1 or 2 μ to 10 μ or more. The same variability held true with the length of individual cells, from a few μ to 40 μ or more. The hyphae were constricted at the septa. In older portions of the mycelium there were short, swollen, thick-walled, apical or intercalary cells which had the appearance of chlamydospores. In some instances, another sort of growth appeared at the margin of the colony. Here the hyphae appeared hyaline, rather short and arranged in finger-like patterns (Fig. 10). Aside from these distortions, young hyphae did not show any of the lytic or degenerative characteristics described above. Preparations stained with Giemsa showed several (up to 11) nuclei in each cell (Fig. 12), and the protoplasmic content was intact and stained uniformly.

The normal fungus sporulated abundantly on PDA. The conidia were 1- to 6-celled and measured 21-49 x 11-17 μ at maturity. It has been shown that in 1, 2, and 3-week old cultures of diseased
Figure 9. Diseased *H. victoriae* obtained by subculturing from severely diseased mycelium and stained with hematoxylin.

Figure 10. Same as Figure 9 but shows a different pattern of growth of mycelium at the margin of the colony.
Figure 11. Diseased *H. victoriae* obtained by subculturing from severely diseased mycelium and stained with giemsa.

Figure 12. Diseased *H. victoriae* obtained by subculturing from severely diseased mycelium, stained with giemsa, and showing nuclei.
H. victoriae conidial production was far below that of the normal fungus (38). Conidia from diseased colonies used in this study were oval in shape, 1- to 2-celled, and measured 14-28 x 10-14 u.

**Respiratory studies of normal and diseased H. victoriae**

Three experiments were carried out with 10-day old mycelial mats from stationary cultures of normal and diseased H. victoriae with 3 replicates in each treatment (Table 1). The respiratory rate in the absence of an external substrate (endogenous respiration) as well as that in the presence of 0.02 M sucrose has been expressed by the amount of oxygen taken up on both the basis of dry weight and nitrogen content of the mycelium. The respiratory rate of the diseased fungus has also been tabulated as percent of that of the normal fungus. Dry weight and nitrogen content determinations were made at the end of each experiment on the samples used. Respiration corresponding to the oxidation of sucrose has been calculated by subtracting the oxygen uptake in the absence of any substrate from the oxygen uptake over the same interval in the presence of sucrose. This is a conventional, widely used method. However, its accuracy is considered questionable, since the possible effect of the internal substrate on the oxidation of the external substrate or the possible influence of the external substrate on the endogenous respiration is not yet definitely known (11, 63).

In each experiment the respiratory rate of diseased H. victoriae both endogenous and in the presence of sucrose, was higher than that of
Table 1. Respiratory rate of 10-day old mycelium of normal (SN-l) and diseased (SD-1) *H. victoriae* from stationary cultures.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Respiration in the absence of an external substrate (endogenous)</th>
<th>Respiration with sucrose as external substrate</th>
<th>Respiration corresponding to the oxidation of sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SN-l</td>
<td>SD-1</td>
<td>% SN-1</td>
</tr>
<tr>
<td>1</td>
<td>4.7</td>
<td>185</td>
<td>5.9</td>
</tr>
<tr>
<td>2</td>
<td>5.6</td>
<td>196</td>
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<tr>
<td>3</td>
<td>4.4</td>
<td>148</td>
<td>5.1</td>
</tr>
<tr>
<td>Ave.</td>
<td>4.9</td>
<td>176</td>
<td>5.9</td>
</tr>
</tbody>
</table>

\[1/\text{Q}_{O_2} = \text{ul O}_2/\text{mg dry weight/hour}\]

\[2/\text{Q}_{O_2}(N) = \text{ul O}_2/\text{mg mycelial nitrogen/hour}\]
normal cultures. On the average, endogenous respiration of the
diseased fungus exceeded that of the normal fungus by about 20 per
cent. There was, however, considerable fluctuation in the respiratory
rates of both the normal and the diseased fungus, for example the
diseased fungus in the third experiment had lower respiratory rates
than that of the normal fungus in the second experiment. In a similar
manner, the data for respiration corresponding to the oxidation of
sucrose were not comparable among experiments. The respiratory rate
of the normal fungus was higher than that of the diseased fungus in the
first and third experiments, whereas the opposite effect was observed
in the second experiment. Statistical analysis gave L.S.D. (0.05) =
1.67 (data on dry weight basis), or L.S.D. (0.05) = 40 (data on basis
of nitrogen content) for comparison with differences between any two
averages listed in Table 1.

It was thought that such variability might be due, at least in part,
to differences in the metabolism of submerged versus aerial mycelium
in stationary cultures, since it was very unlikely that samples of
uniform composition were obtained (11). Accordingly, all subsequent
experiments were carried out by using mycelial pellets from shake-
cultures. In these experiments, 0.01 M dextrose was used as an
external substrate. The respiratory rate corresponding to the oxidation
of this substrate was calculated as in the previous experiments. Dry
weights and nitrogen contents were determined from aliquots at the
start of the experiment.

To trace the respiratory rate of normal and diseased *H. victoriae* during a period of 10 days after transplanting, respiratory measurements, based on dry weights (Table 2), were made every second day. The experiment was performed twice with four replicates for each treatment. Average values of the two experiments have been plotted (Fig. 13) and the data of the two experiments were combined and analyzed statistically as a factorial-type experiment. This analysis gave L.S.D. (0.05) = 1.74 and L.S.D. (0.01) = 2.35 for comparison with differences between any two averages listed in Table 2 or plotted in Figure 13. As it is shown in Figure 13, the rates for endogenous respiration and that in the presence of dextrose of both normal and diseased *H. victoriae* were high up to the fourth day after transplanting. After the fourth day the respiratory rates dropped rather abruptly. From the second through the sixth day, the respiratory rate of the diseased fungus was constantly higher than that of the normal fungus. After the sixth day, the respiratory rates of the normal and the diseased fungus tended to approach each other, and from the eighth until the tenth day the rates were more or less the same. The respiratory rates corresponding to the oxidation of dextrose followed a different pattern; a rise occurred on the sixth and the eighth days after transplanting. From the sixth until the tenth day, the respiratory rate of the normal fungus was higher than that of the diseased fungus. This might be explained by the fact that during the period when the decrease in the rates of endogenous respiration of the normal and the diseased
Table 2. Respiratory rate of normal and diseased *H. victoriae* from shake-cultures during a 10-day period after transplanting.

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Exp.</th>
<th>Days after transplanting</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>Days after transplanting</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>Days after transplanting</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Fungus</td>
<td></td>
<td>Respiration in the absence of an external substrate (endogenous respiration)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Respiration with dextrose as an external substrate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Respiration corresponding to the oxidation of dextrose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10.5</td>
<td>11.1</td>
<td>8.8</td>
<td>7.8</td>
<td>7.5</td>
<td>13.0</td>
<td>13.2</td>
<td>12.5</td>
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<td>3.7</td>
<td>4.8</td>
<td>2.3</td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>11.1</td>
<td>10.9</td>
<td>9.2</td>
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<td>6.8</td>
<td>14.7</td>
<td>13.0</td>
<td>12.7</td>
<td>10.1</td>
<td>7.9</td>
<td>3.6</td>
<td>2.1</td>
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<td>2.9</td>
<td>1.1</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Average</td>
<td>10.8</td>
<td>11.0</td>
<td>9.0</td>
<td>7.5</td>
<td>7.1</td>
<td>13.8</td>
<td>13.1</td>
<td>12.6</td>
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<td>3.6</td>
<td>3.6</td>
<td>1.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diseased Fungus</td>
<td></td>
<td></td>
<td>13.4</td>
<td>12.9</td>
<td>11.2</td>
<td>8.4</td>
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<td>15.5</td>
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<tr>
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<td>12.6</td>
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<td>6.5</td>
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<td>2.3</td>
<td>2.6</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Average</td>
<td>13.0</td>
<td>13.1</td>
<td>11.1</td>
<td>7.4</td>
<td>7.0</td>
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<td>15.6</td>
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<td>8.1</td>
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<td>3.1</td>
<td>2.5</td>
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<td></td>
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<tr>
<td>Average change of diseased fungus when average for normal fungus was 100</td>
<td>120</td>
<td>119</td>
<td>123</td>
<td>99</td>
<td>98</td>
<td>123</td>
<td>119</td>
<td>113</td>
<td>88</td>
<td>92</td>
<td>83</td>
<td>109</td>
<td>89</td>
<td>72</td>
<td>59</td>
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Figure 13. Respiratory rate of normal and diseased H. victoriae from shake-cultures during a 10-day period after transplanting.
fungus were comparable, the decrease in the respiration rate of the normal fungus in the presence of dextrose was smaller than that of the diseased fungus.

In addition, the averages of the respiratory rates of the diseased fungus have been expressed in per cent of those of the normal fungus (Fig. 14).

Seven experiments were carried out to trace the respiratory response of mycelial pellets from 4-day old cultures of normal and diseased H. victoriae to different concentrations of 2,4-dinitrophenol (DNP). Two or three replicate respiratory vessels were used for each treatment in each experiment, and rates were determined on the basis of both dry weight and nitrogen content (Tables 3 and 4). In three experiments, the R.Q. of endogenous respiration and that in the presence of dextrose of both the normal and the diseased fungus was also determined (Tables 3 and 4). Mean values for DNP dosages have been plotted in Figures 15 and 16. To evaluate differences between any two of these means, the data were analyzed statistically. This analysis gave the following L.S.Ds:
Figure 14. Deviation of respiratory rates of diseased *H. victoriae* from those of the normal fungus during a 10-day period after transplanting.
Table 3. Effect of DNP on the respiratory rates (ul O₂/mg dry weight/hour) of 4-day old, shake-cultures of normal and diseased H. victoriae.

<table>
<thead>
<tr>
<th></th>
<th>Normal Fungus</th>
<th></th>
<th>Disease Fungus</th>
<th></th>
</tr>
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<td>Concentrations of DNP in molarity</td>
<td></td>
<td>Concentrations of DNP in molarity</td>
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<tr>
<td>Exp.</td>
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<td>Cont. 5x10⁻⁶ 1x10⁻⁵ 5x10⁻⁶ 1x10⁻⁴ 5x10⁻³</td>
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<tr>
<td></td>
<td>R.Q.</td>
<td></td>
<td>R.Q.</td>
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<td>Respiration in the absence of an external substrate (endogenous respiration)</td>
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<td>10.8 11.0 11.3</td>
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</tr>
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<tr>
<td>%</td>
<td>Cont. 100 100 97 138 121 24</td>
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Continued
Table 3. Continued

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<th>Exp.</th>
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<th></th>
<th>Disease Fungus</th>
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<th></th>
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</thead>
<tbody>
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<td>Concentrations of DNP in molarity</td>
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<td></td>
<td>Concentrations of DNP in molarity</td>
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<tr>
<td></td>
<td>Cont.</td>
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<td>14.4</td>
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<tr>
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<td>11.75</td>
<td>11.70</td>
<td>15.22</td>
<td>13.10</td>
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<tr>
<td>%</td>
<td>Cont.</td>
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<td>91</td>
<td>118</td>
<td>101</td>
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Continued
Table 3. Continued

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<th>Cont.</th>
<th>5x10^-6</th>
<th>1x10^-5</th>
<th>5x10^-6</th>
<th>1x10^-4</th>
<th>5x10^-4</th>
<th>R.Q.</th>
<th>Cont.</th>
<th>5x10^-6</th>
<th>1x10^-5</th>
<th>5x10^-6</th>
<th>1x10^-4</th>
<th>5x10^-4</th>
<th>R.Q.</th>
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<td>1.3</td>
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<td>4.1</td>
<td>2.6</td>
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<td>Ave.</td>
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<td>1.65</td>
<td>0.85</td>
<td>0.57</td>
<td>0.60</td>
<td></td>
<td>2.77</td>
<td>1.30</td>
<td>1.25</td>
<td>1.17</td>
<td>0.43</td>
<td>0.00</td>
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</tr>
<tr>
<td>%</td>
<td>Cont.</td>
<td>100</td>
<td>51</td>
<td>65</td>
<td>33</td>
<td>22</td>
<td>24</td>
<td>100</td>
<td>47</td>
<td>45</td>
<td>42</td>
<td>15</td>
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Table 4. Effect of DNP on the respiratory rates (ul O₂/mg nitrogen/hour) of 4-day old, shake-cultures of normal and diseased *H. victoriae*.

<table>
<thead>
<tr>
<th></th>
<th>Normal Fungus</th>
<th></th>
<th>Diseased Fungus</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Concentrations of DNP in molarity</td>
<td></td>
<td>Concentrations of DNP in molarity</td>
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</tr>
<tr>
<td>Exp.</td>
<td>Cont. 5x10⁻⁶  1x10⁻⁵  5x10⁻⁴  1x10⁻³  5x10⁻¹  R.Q.</td>
<td></td>
<td>Cont. 5x10⁻⁶  1x10⁻⁵  5x10⁻⁴  1x10⁻³  5x10⁻¹  R.Q.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Respiration in the absence of an external substrate (endogenous respiration)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>228 217 312</td>
<td>258 263 271</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>242</td>
<td>284 59 0.80</td>
<td>313</td>
<td>284 42 1.02</td>
</tr>
<tr>
<td>3</td>
<td>236 226</td>
<td>59 0.88</td>
<td>316 322</td>
<td>75 0.97</td>
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<td>4</td>
<td>231 319</td>
<td>0.96</td>
<td>330 307</td>
<td>1.03</td>
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<tr>
<td>5</td>
<td>219 229</td>
<td>284</td>
<td>291 299</td>
<td>297</td>
</tr>
<tr>
<td>6</td>
<td>236 338</td>
<td>331 273</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>235 242 298</td>
<td>259</td>
<td>306 280 310 283</td>
<td>275 58</td>
</tr>
<tr>
<td>Ave.</td>
<td>232 229 227</td>
<td>317 276 59</td>
<td>306 280 310 283</td>
<td>275 58</td>
</tr>
<tr>
<td>Exp.</td>
<td>Normal Fungus</td>
<td>Diseased Fungus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>------</td>
<td>--------------</td>
<td>----------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Concentrations of DNP in molarity</td>
<td>Concentrations of DNP in molarity</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cont. 5x10^-6 1x10^-5 5x10^-6 1x10^-4 5x10^-4 R.Q.</td>
<td>Cont. 5x10^-6 1x10^-5 5x10^-6 1x10^-4 5x10^-4 R.Q.</td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>275 255 330</td>
<td>313 299 274</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>284 308 62 1.00</td>
<td>343 204 35 1.06</td>
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<td>3</td>
<td>283 258 78 1.11</td>
<td>432 376 59 1.07</td>
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</tr>
<tr>
<td>4</td>
<td>318 348 1.04</td>
<td>435 372 1.04</td>
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</tr>
<tr>
<td>5</td>
<td>283 271 289</td>
<td>373 317 300</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>303 350</td>
<td>362 289</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>280 267 306 269</td>
<td>352 325 314 259</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ave.</td>
<td>289 261 264 333 289 70</td>
<td>373 312 346 312 284 47</td>
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</tbody>
</table>

Respiration in the presence of 0.01 M dextrose
Table 4. Continued

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Normal Fungus Concentrations of DNP in molarity</th>
<th>Diseased Fungus Concentrations of DNP in molarity</th>
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<tbody>
<tr>
<td></td>
<td>5x10^{-6}</td>
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<tr>
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<td>7</td>
<td>45</td>
<td>25</td>
</tr>
<tr>
<td>Ave.</td>
<td>57</td>
<td>31</td>
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</tbody>
</table>

Respiration corresponding to the oxidation of dextrose
A study of Figures 15 and 16 shows that the patterns of respiratory rates of both the normal and the diseased fungus on dry weight basis did not differ appreciably from those on nitrogen basis. The endogenous respiratory rate of the normal fungus as well as that observed in the presence of dextrose showed a considerable increase with DNP at a concentration of $5 \times 10^{-5}$ M. This increase diminished when DNP was used at a concentration of $1 \times 10^{-4}$ M. With further increase in the concentration of DNP, the respiratory rates dropped rather abruptly. In contrast, the analogous respiratory rates of the diseased fungus showed a constant and gradual depression in the presence of DNP at concentrations $1 \times 10^{-5}$, $5 \times 10^{-5}$, and $1 \times 10^{-4}$ M. This depression was statistically insignificant for endogenous respiration, but was significant for respiration in the presence of dextrose. Concentrations of DNP above $1 \times 10^{-4}$ M resulted in respiratory rates of the diseased fungus that paralleled very closely those of the normal fungus.

In Table 3, averages of the respiratory rates with different concentrations of DNP have also been expressed as per cent of controls (Fig. 17).
Figure 15. Effect of DNP on the respiratory rates of 4-day old, shake-cultures of normal and diseased H. victoriae.
Figure 16. Effect of DNP on the respiratory rates of 4-day old, shake-cultures of normal and diseased *H. victoriana*. 
Figure 17. Deviation of respiratory rates of normal and diseased H. victoriae from controls in the presence of DNP.
The R.Q. of the diseased fungus, at all times very close to 1 both in the presence and in the absence of dextrose, suggested a possible predominance of carbohydrates in respiration. The R.Q. of the normal fungus in endogenous respiration was below 1, but reached the value 1 after the addition of dextrose. This might indicate a predominant participation of proteins and/or fatty acids in the endogenous respiration of this fungus.

In these experiments, dry weights and nitrogen contents were determined on the same samples of 10-day old, stationary, and 4-day old, shake-cultures. The nitrogen content has been expressed as per cent of the dry weight (Table 5). Although the average for the diseased fungus was slightly lower than that for the normal fungus in both stationary and shake-cultures, the range of variability among values was so great that the difference between these averages was not appreciable. This was not the case, however, with the difference between stationary and shake-cultures. The nitrogen content of the shake-cultures was almost 50 per cent greater than that of the stationary cultures.

Study of infection of oats by normal and diseased H. victoriae

Lindberg (38) has found that the pathogenicity and toxin production of diseased H. victoriae was markedly lower than that of the normal fungus. The comparison of pathogenicity was based on the number of plants that survived inoculation. There was a need to study the mode of infection and early disease development caused by normal and
Table 5. Nitrogen content of normal and diseased *H. victoriae* expressed in percentage of dry weight.

<table>
<thead>
<tr>
<th>Stationary Cultures</th>
<th>Shake Cultures</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Normal Fungus</td>
</tr>
<tr>
<td>1</td>
<td>2.39</td>
</tr>
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<td>2</td>
<td>2.84</td>
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<tr>
<td>3</td>
<td>2.48</td>
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<td>8</td>
<td>3.21</td>
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<tr>
<td>9</td>
<td>3.05</td>
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<tr>
<td>Average</td>
<td>2.84</td>
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</table>
diseased *H. victoriae* in susceptible Victor-grain 48-93 oats. Several inoculation tests were performed on seedlings in the greenhouse to study this aspect of the problem.

Six hours after inoculation of leaves with normal *H. victoriae*, slender, thin-walled, hyaline hyphae developed along the depressions between epidermal cells. These hyphae remained unbranched and non-septate until approximately nine hours after inoculation. At that time, in about half the inoculation sites, the hyphae had penetrated the leaf at several points. In the majority of instances, penetration occurred directly between epidermal cells (Figs. 18, 19, 20) and less frequently through the epidermal cells. Each of these cases of penetration was usually accompanied by the formation of an appressorium. In some instances, penetration also took place through the stomates with or without the formation of an appressorium (Figs. 20, 21). Twelve hours after inoculation, many penetrations were found in almost all sites of inoculation. Following penetration, the predominant growth of the fungus was intercellular, but in a few instances intracellular hyphae were also observed. At 18 hours, the fungus at a few inoculation sites had completely traversed the leaf blade from the upper to the lower surface, and at 24 hours such advance of the fungus was common. Upon reaching the lower epidermis, the fungus grew out first through the stomates (Fig. 22) and later between epidermal cells. It was at this stage that the first signs of reaction of the host cells appeared. The reaction was a
Figure 18. Penetration of Victor-grain 48-93 oat leaves by *H. victoriae* through and between epidermal cells.

Figure 19. Penetration of Victor-grain 48-93 oat leaves by *H. victoriae* through and between epidermal cells.
Figure 20. Penetration of Victor-grain 48-93 oat leaves by H. victoriae through stomates.

Figure 21. Penetration of Victor-grain 48-93 oat leaves by H. victoriae through stomates.
Figure 22. *H. victoriae* growing out through stomate of the lower leaf epidermis of Victor-grain 48-93 oats.

Figure 23. *H. victoriae* forming abundant conidiophores on the lower leaf epidermis of Victor-grain 98-93 oats.
yellowish discoloration of the tissue and a reduction in chlorophyll-content followed by collapse of the host cells. This reaction was erratic, however, since cellular degeneration was more frequent at a distance from the advancing fungus rather than close to it. At the same time, where mycelium was abundant in the leaf tissue, the first young conidiophores developed between the lower epidermal cells and sometimes from the mycelium found on the surface of the lower epidermis.

By 36 hours after inoculation, numerous conidiophores and a few young conidia had developed (Fig. 23). Twelve hours later, the above phenomena had intensified, and the fungus covered large areas in which the host tissue had collapsed. Numerous conidiophores with fully developed conidia were found in these areas. In the following 24-hour period, the decomposition of the host tissue was so advanced as to lead to the formation of holes through the sites of inoculation. As long as the host tissue remained intact the fungus advanced very little in the longitudinal plane. Later, however, as the leaf became chlorotic, and then necrotic, the fungus advanced from the inoculated area to a distance of one cm or more toward the leaf tip, and downwards to cover the leaf sheath and reach the culm.

Although there were considerable differences among inoculation tests with diseased *H. victoriae*, and even from one to another inoculation site of the same test, the general pattern of infection was comparable with that outlined for normal *H. victoriae*. The differences
observed between diseased *H. victoriae* and normal *H. victoriae* were quantitative rather than qualitative. Difference occurred in the percentage of penetrations, which were relatively few in the case of the diseased fungus as compared with the normal fungus. It has been shown that necrotic lesions developed at the sites of inoculation with either the normal or the diseased fungus (38). The lesions were followed by necrosis of the inoculated leaf. Later, however, the development of Victoria blight in plants inoculated with the diseased fungus was markedly less. Another difference was in the tendency for sporulation. While at about 48 hours after inoculation, sporulation of the normal fungus was common, sporulation was found to take place only in approximately 30 per cent of the leaves inoculated with the diseased fungus. Difference was also observed in the degree of the reaction of the host tissue. The degeneration of the inoculation sites with the diseased fungus was always less as compared with tissue infected with the normal fungus. This was illustrated by the fact that tissue infected with the normal fungus became difficult to stain and examine microscopically by 72 hours after inoculation, whereas tissue infected with the diseased fungus was firm enough even 120 hours after inoculation.

Paddock\(^1\) has studied thoroughly the process of infection of susceptible and resistant oat varieties with *H. victoriae*. He inoculated

leaves by dusting them with a mixture of conidia and talc. The pattern of infection of susceptible Vicland oats described by him does not differ appreciably from the pattern of infection described above for Victor-grain oats inoculated with the normal fungus. Notable differences exist, however, as regard to the time intervals at which the main steps of the infection process took place. For example, although not giving the exact time at which penetration occurred, Paddock has noticed that the fungus had traversed the leaf 24 hours after inoculation, and begun to form conidiophores between 36 and 48 hours and a few conidia at the latter time. In the present study, normal H, victoriae had penetrated the leaf epidermis 9 hours after inoculation. At 18 hours, the fungus had traversed the leaf completely and grew out from the lower epidermis. The fungus formed the first conidiophores 24 hours after inoculation, and the first conidia were observed 12 hours later. Paddock's description of the disintegration of the host tissue following infection also shows that such reaction of the host was considerably slower and less intensive, compared with that observed in the present study. From the foregoing it seems reasonable to believe that the faster progress of the fungus and the more intensive host reaction observed in this study were due mainly to the method of inoculation used. Differences in environmental conditions as well as in the virulence of the fungus or the sensitivity of the host might, however, have contributed to the differences observed.
DISCUSSION

Two reasonable explanations for the nature of the cause of the disease of *Helminthosporium victoriae* are that it is an infectious agent, such as a virus, or that it is a heritable phenomenon. Previous attempts to identify an infectious causal agent by transmission studies with cell-free preparations of the diseased fungus have not been successful (36, 39). Studies on the inheritance of the disease have not been done and such studies are complicated by heterokaryosis in *Helminthosporium*. Consequently, comparative studies of normal and diseased *H. victoriae* have been emphasized with the hope of shedding light on the nature of the causal agent of the disease. The gross cultural symptoms of the disease in *H. victoriae* have strongly indicated deleterious effects on the fungus and such effects were found to be equally expressed in microscopic irregularities. A comparison of the morphology and cytology of normal and diseased *H. victoriae* have revealed important differences. Marked differences were also observed in the morphology and cytology of newly diseased colonies as compared to colonies obtained by sub-culturing from severely diseased *H. victoriae*. Cells of the latter did not show signs of lytic degeneration and abundant nuclei were present. Except for size of the cells and the pattern of growth of the mycelium, few differences were observed between these cultures and normal cultures of the fungus. The protoplasmic content, however, of young
The hyphae of newly diseased colonies showed various degrees of degeneration. The cells were often completely devoid of protoplasm and, even where protoplasmic materials were present, attempts to detect nuclei were unsuccessful. The loss of protoplasm from young hyphal cells of newly diseased *H. victoriae* might indicate an increased permeability on the cell wall. The osmotic effect of the killing, fixing, and staining materials of such cells, however, might have contributed to the differences that were observed. It has also been observed that the spread of the disease was favored in young immature hyphae, while the older, differentiated cells of the same colony might be unaffected (36). These differences have led us to regard the disease condition in newly diseased colonies as one of an acute nature, while that in cultures propagated by subculturing severely diseased *H. victoriae* as one of a chronic nature.

In general, metabolic studies in fungi showing any kind of abnormalities have been limited. Nevertheless, in the few cases studied, inhibition of growth due to genetic or fungicidal factors appeared to be associated with a reduced respiratory rate (11, 14, 20, 21, 61, 62, 72). Diseased *H. victoriae* with markedly inhibited growth showed a respiratory rate higher than that of the normal fungus. This was observed in 10-day old, stationary cultures and in up to 6-day old, shake-cultures. This metabolic alteration resembled an analogous response of higher plant tissue following infection (2, 18, 41, 64, 68, 69, 70). In addition, diseased *H. victoriae* shared another metabolic feature characteristic
of infected plant tissue (1, 16, 18, 56, 64). Diseased *H. victoriae* did not show any increase in respiratory rate at low concentrations of DNP, such as 5x10^-5 and 1x10^-4 M, at which normal *H. victoriae*, as was expected, showed a positive response. These metabolic alterations of the diseased fungus might be interpreted as suggesting an infectious nature for the disease.

The comparative study of infection of seedlings of susceptible oats revealed little differences between the pattern of infection with diseased *H. victoriae* and that with the normal fungus. These differences were quantitative rather than qualitative. There was, however, a considerable difference in the degree of the host reaction to infection between tissue infected with the normal fungus and tissue infected with the diseased fungus. The degeneration of tissue infected with the normal fungus was faster and more extensive than that of tissue infected with the diseased fungus. It has been shown that, while development of lesions and necrosis of the inoculated leaves followed inoculation with either normal or diseased *H. victoriae*, the development of Victoria blight in plants inoculated with the diseased fungus was markedly less than that with the normal fungus (38). It has also been shown that the toxin production by the diseased fungus was markedly less than that of the normal fungus (38). These findings seem to suggest that the lower pathogenicity of diseased *H. victoriae* as compared with that of the normal fungus might be due mainly to reduced toxin production.
SUMMARY

In comparative morphological cytological studies of normal and diseased colonies of *Helminthosporium victoriae*, cellophane-fungus preparations were stained with three different dyes and examined microscopically. In contrast to the normal cultures, diseased colonies exhibited a variety of irregularities. Young hyphae from newly diseased colonies showed different degrees of lytic degeneration, and attempts to detect nuclei in such hyphae were unsuccessful. Young hyphae from colonies obtained by subculturing from severely diseased mycelium, on the other hand, did not show any signs of lytic degeneration and contained abundant nuclei. These hyphae did, however, show a variety of distortions in the morphology of cells and in the pattern of growth.

In comparative respiratory studies of normal and diseased cultures of *H. victoriae*, diseased colonies showed certain alterations. Diseased colonies from 10-day old stationary cultures and from 2- to 6-day old shake-cultures showed an increased rate in both the endogenous respiration and that in the presence of dextrose, compared to normal colonies. Diseased colonies from 4-day old shake-cultures did not show any respiratory increase in the presence of low concentrations of DNP (5x10^{-5} and 1x10^{-4} M), whereas normal colonies showed a positive response. While the R.Q. for the normal fungus in endogenous respiration was below 1 and reached the value 1 with the addition of dextrose,
the R.Q. for the diseased fungus was around 1 either in the presence or in the absence of dextrose.

Comparative study of the process of infection of susceptible oat seedlings with normal and diseased *H. victoriae* revealed little differences. However, the degeneration of tissue inoculated with the diseased fungus was slower and less extensive than that of tissue inoculated with the normal fungus.


Evangelos E. Psarros was born in Neapolis-Vion, Laconia, Greece, on October 21, 1917. He received his elementary education at the elementary school of his native town and his secondary education at the Gymnasium of Cythera, Greece. He attended the Averoff Agricultural School, Larissa, Greece, and was graduated from this school in 1937. He served for three times in the Greek Army. In July 1946, he was employed by the Ministry of Agriculture of Greece. In October 1948, he enrolled in the Agricultural College of Athens, Greece, and received his Diploma from that institution in-February 1954. While employed by the Ministry of Agriculture, he served after his graduation at the Phytopathological Station of Patras, Greece, and the Plant Pathology Laboratory of Agricultural College of Athens, Greece. He began graduate study in the Department of Botany, Bacteriology and Plant Pathology at Louisiana State University in September 1957, and received the degree of Master of Science in May of 1959. He continued graduate study at the same Department and is now a candidate for the degree of Doctor of Philosophy.
EXAMINATION AND THESIS REPORT

Candidate: Evangelos E. Psarros

Major Field: Plant Pathology

Title of Thesis: Comparative Morphology and Respiration of Normal and Diseased Helminthosporium victoriae

Approved:

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

M. T. Henderson

Lewis H. Flint

B. Henry

H. E. Wheeler

St. John P. Chilton

Date of Examination:

November 28, 1960