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Effects of Light on Production of Toxins by *Helminthosporium victoriae* and *H. carbonum*

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Abstract

Helminthosporium victoriae and *H. carbonum* were grown under fluorescent plus incandescent lights, or in darkness, with several different temperature regimes. There was little or no effect of light on toxin production by *H. victoriae*. Light-grown cultures of *H. carbonum* had significantly higher titres of host-specific toxin than did dark-grown cultures.

Light had no significant effect on growth of either fungus. Maximum titres of host-specific toxins from both fungi were evident at the time maximum growth was reached. Minimum pH values in Fries modified medium occurred at the time of, or slightly before maximum level of toxin was reached.

Introduction

At least ten species of fungi are now known to produce toxins which affect host but not non-host plants. Among the known producers of such "host-specific toxins" are *Helminthosporium victoriae* Meehan & Murphy, cause of a blight disease of oats, and *H. carbonum* Ullstrup, cause of a leaf-spot disease of maize (8). These two fungi were examined for possible effects of light on production of their respective host-specific toxins.

We had previous information that *H. maydis* race T produces more toxin in the light than in the dark (J.M. Daly, unpublished).

Several environmental conditions are known to affect production of host-specific toxins by *H. victoriae* and *H. carbonum*. Very little toxin is produced in aerated cultures, but yields are good in stationary cultures grown at 21-25°C. The best media for toxin production are well-buffered in the acid range; a modified Fries solution is usually used. *H. carbonum* produces significant amounts of toxin only when yeast extract is added to Fries medium; most isolates of *H. victoriae* do not require this supplement.

Higher toxin titres are reached in small volume than in large volume cultures of both fungi. Maximum toxin concentrations in culture occur shortly after the peak of growth is reached; toxin then decreases as the cultures lyse and the pH rises(4, 5). Isolates of both fungi vary greatly in potential for toxin production(5, 7), and this ability is often lost in culture.

Materials and Methods

H. victoriae isolate LN and *H. carbonum* race 1 were grown in Roux bottles, each containing 150 ml of Fries modified solution. The medium contained, in g/liter: ammonium tartrate, 5.0; NH_4NO_3, 1.0; KH_2PO_4, 1.0; $MgSO_4 \cdot 7H_20$, 0.5; NaCl, 0.1; $CaCl_2 \cdot 2H_2O$, 0.13; sucrose, 30.0; and yeast extract, 1.0. Cultures were incubated at room temperature(approximately 23°C) or in controlled growth chambers, under fluorescent plus incandescent lights.

Some cultures in each experiment were wrapped with aluminum foil to exclude light. Dry weights were taken at harvest time; cultures were filtered through tared Whatman No. 1 filter paper and the

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fungal mats were dried at 95°C for 48 hours. Filtrates from at least three Roux bottle cultures were combined and serial dilutions were made for toxin bioassays.

The seedling root assay (5) was used for *H. victoriae* toxin. Glumes were removed from susceptible (cv. Park) and from resistant (cv. Garry) oat seeds, which were then incubated between sheets of moist filter paper for 24 hours prior to toxin exposure. Five ml of each dilution of toxic culture filtrate were added to each of four petri plates (5.0cm); five germinated susceptible seeds were placed in each of two plates, and five resistant seeds were placed in each of two plates. Dilution end-points of the assay were determined after the seedlings had grown for two days; the end-point was the maximum dilution that prevented roots from growing more than 1.0cm. Control roots in water were 5.0 cm in length.

Seeds of corn hybrids that are susceptible (Pr X K61) and resistant (Pr 1 X K61) were used for bioassay of *H. carbonum* toxin, as described elsewhere (6, 7). Five seeds per petri dish (9cm) were germinated in 10 ml of White's nutrient solution for 24 hours prior to addition of toxin. Serial dilutions of toxin containing filtrates were used; the toxin dilution end-point was the maximum dilution that inhibited root growth by 50 per cent.

Results

For the first experiment, *H. victoriae* was grown at room temperature (approximately 23°C). One third of the cultures were grown under continuous light, another third had a 16 hours per day light period, and a final third of the bottles were wrapped with foil to exclude light. Cultures in the light had a slightly higher temperature (approximately 1°C) than did cultures in the dark.

Maximum growth was reached in 9 to 14 days; there appeared to be no effect of light on total dry weight, although the cultures in continuous light appeared to achieve maximum growth somewhat earlier than did the others (Fig.1,A). This could have resulted the slightly higher temperature. The pH values of culture filtrates differed somewhat; minimal pH was reached by cultures in continuous light at day 9(pH4.0), by cultures with 16 hours light at day 14 (pH 3.2) and cultures without light at day 9 (pH 3.6). In all cases, the pH values for culture filtrates increased rapidly after the low was reached (Fig.1,B). Maximum toxin titres for all treatments were obtained at 14 days, after which toxin titres decreased significantly (Fig. 1,C). Light had no effect on toxin titre during the first 14 days, but cultures in light gave slightly higher assays at day

Table 1. Effect of light on growth, pH, and toxin production by *Helminthosporium victoriae* under controlled condition

Treatment	Time(days)	Dry wt. (mg)	pH	Toxin assay (Dilution End Point)
1) Light, 15hr at 26±1°C	10	723	4.8	100
Dark, 9hr at 18±1°C	14	1,249	4.2	1,600
	18	1,548	3.9	25,600
	25	1,196	7.3	1,600
2) Continuous dark	10	1,035	4.3	100
15hr at 26°C	14	1,471	3.7	6,400
9hr at 18°C	18	1,639	3.3	6,400
	25	1,519	5.9	6,400
3) Light, 15hr at 22±1°C	10	—	—	400
Dark, 9hr at 21±1°C	14	—	—	25,600
	18	—	—	409,600
	25	—	—	6,400

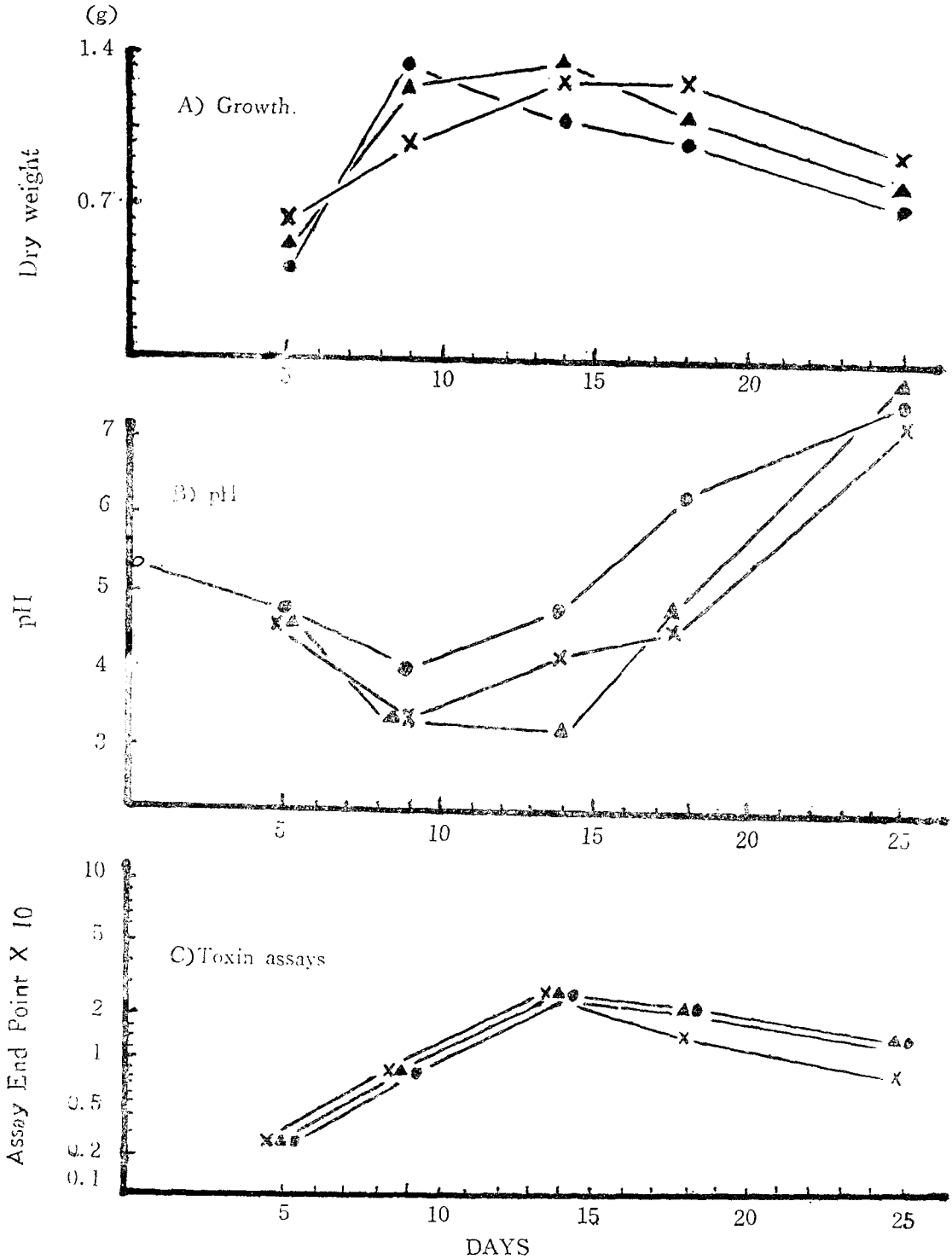

Fig.1. Effect of light on growth, pH and toxin production by *Helminthosporium victoriae* at room temperature. Three conditions were used: continuous light(●……●), light for 16 hours/day, (▲——▲), and continuous darkness(×……×) at approximately 23°C. Each value is the average for 3 cultures.

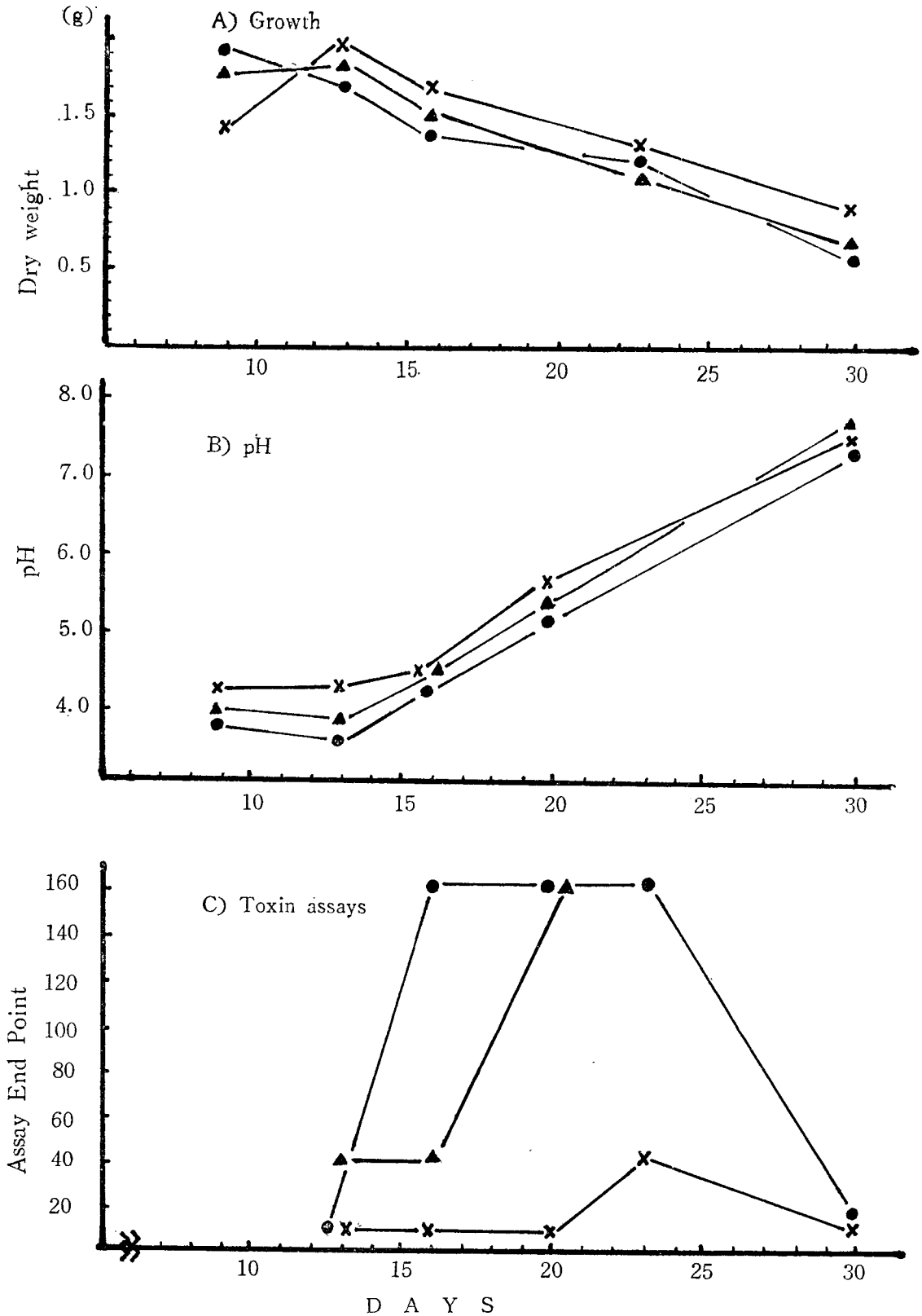

Fig.2. Effects of light on growth, pH, and toxin production by *Helminthosporium carbonum* at room temperature. Cultures were grown on the laboratory bench under continuous light at approximately 24°C (●……●), under 15 hours light per day at 22-24°C (▲……▲), and without light at approximately 22°C (×……×). Each value is the average for 3 cultures.

18 and 25. Such difference could have resulted from more rapid toxin inactivation in one case than in the other.

In a second experiment, temperatures were controlled more precisely by incubating the *H. victoriae* cultures in controlled chambers. One set of cultures was given 15 hours light per day, with the temperature at 26±1°C during the light period and 18±1°C during the dark period. A second set of cultures was held in the growth chamber at the same alternating temperatures but without light. A third set of cultures was incubated under lights for 15 hours per day at 22±1°C, and without light for 9 hours at 21±1°C.

Maximum growth and minimum pH values under all these conditions were reached in 18 days. Also, cultures grown with light had maximum toxin titres at 18 days (Table1). Light did not appear to have a consistant or significant effect on toxin production in the cultures grown at 26/18°C, but the cultures grown with alternating light and darkness at 22/21°C had higher toxin titres (Table 1). These data, although not conclusive, indicate that temperature affects toxin production more than does light.

H. carbonum race 1 was grown at room temperature with three different treatments: (a) some cultures were kept under continuous light, with the temperature of the culture at approximately 24°C; (b) other cultures were held under lights for 15 hours per day, with an average temperature approximately 23°C; and (c) still other cultures were grown without light, with the temperature approximately 22°C. Dry weig ht, pH and toxin titre were monitored at intervals, using three Roux bottle cultures per assay.

Maximum dry weight was reached by day 9 in cultures with light, and by day 13 in cultures without light (Fig.2, A). pH values for all treatments rose steadily after 13 days. Toxin titres for cultures in light were higher than for cultures without light (Fig. 2, C). However, the results were suspect because of small differences in temperature.

Toxin production by a different isolate of *H. carbonum* was examined in a second experiment, using more precisely controlled temperatures. Cultures were grown in a controlled chamber with air circulation, under continuous light or in darkness at 21±1°C.

The pH of these cultures reached low values (pH 3.5) by day 15; thereafter, the pH value of cultures in light increased rapidly, whereas the pH of cultures in darkness remained relatively low. The first toxin assays were taken at day 10, when the titre was already relatively high. There was significantly more toxin in lightgrown than in dark-grown cultures until day 18 (Table 2). In contrast to the results with *H. victoriae*, light appears to affect toxin production by *H. carbonum* race 1.

Table 2. Effects of light toxin titre and pH in cultures of *Helminthosporium carbonum* grown under controlled conditions at 21±1°C.

Treatment	Time (days)	pH	Toxin assay (Dilution End Point)	Treatment	Time (days)	pH	Toxin assay (Dilution End Poit)
1) Continuous light	10	4.2	640	2) Darkness	10	4.2	160
	15	3.2	640		15	3.0	160
	16	4.4	2,560		16	3.3	160
	18	5.5	640		18	3.2	640
	23	7.3	640		23	3.9	640

Discussion

Light is know to stimulate growth of *Blastocladiella emersonii* [1], and there are reports of stimulation for certain filamentous fungi. In general, however, light appears to inhibit or to have little effect on growth of filamentous fungi [3] ; we did not demonstrate an effect on growth of *H. victoriae* and *H. carbonum*. On the other hand, light may affect metabolism of fungi in culture, even when no growth res-

ponses are evident. For example, low levels of flavoprotein inhibitors did not change growth of *Phycomyces* in the dark, but gave striking inhibition of growth in light [2]. Light is also known to affect sporulation, pigmentation, and other developmental processes. Action spectra indicate that a flavin may be a common photoreceptor in fungi. One suggestion is that flavin is the photoreceptor for a "fast reaction" in light, and that destruction of flavin is the basis for a "slow adapting reaction" [3] There are no data to suggest that such mechanisms are involved in synthesis of toxins.

Three fungi have now been examined for effects of light on accumulation of host-specific toxins in cultures. Light appears to stimulate toxin production by *H. maydis* race T(J.M. Daly, unpublished) and *H. carbonum* race 1, but does not seem to affect production by *H. victoriae*. Under most conditions, *H. maydis* and *H. carbonum* are leaf-infecting fungi, whereas *H. victoriae* usually invades roots and lower stems. There are not sufficient data for generalizations, but the effect of light on toxin production could be correlated in some way with the ecology of these fungi.

抄 錄

寄生特異的 毒性物質을 生成하는 菌類 중에서 *Helminthosporium victoriae*와 *H. carbonum*을 몇개의 다른 溫度에서 螢光燈과 白熱燈을 利用한 光條件과 暗條件下에서 培養하였다. 그 結果 HV-toxin 生成은 光線의 影響을 거의 받지않았으나 HC-toxin은 暗黑보다 光線條件에서 기른 培養에서 더많이 生成되었다. 그러나, 光線이 菌의 生育에 미치는 影響은 크지 않았으며 단지 菌의 生長이 最大에 到達한 以後에만 毒性物質의 含量이 最大가 되었다. 一般的으로 pH가 最下에 이른 後에 毒性物質 含量이 最大가 되었다.

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