Environmental Factors Regulating Sexual and Asexual Reproduction by Mycosphaerella ligulicola

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ABSTRACT

Cardinal temperatures for formation of pycnidia and perithecia of *Mycosphaerella ligulicola* differed significantly. The temperature/pycnidium-development curve was nearly identical to that for linear growth of the fungus on potato-dextrose agar; i.e., minimum 3, optimum 26, maximum 30 C. Perithecia were not produced at temperatures greater than 24 C; the optimum was 21 C. The effect of temperature on asexual reproduction is compounded by its effect on spore number, size, and septation. Conidium size and per cent of septate conidia produced are inversely proportional to temperature; number of conidia produced is directly related to temperature. Light is required for reproduction by certain isolates, not by others. Isolates requiring light for sporulation are stimulated toward pycnidium formation by near-ultraviolet light at a daily photoperiod of 10 to 15 hr. Perithecium production appears to be stimulted when the proportion of far red light exceeds that in the near red portion of the spectrum at over-all irradiance levels of 450 $\mu w/cm^2$. However, this effect was overridden at a higher light intensity, 1,500 $\mu w/cm^2$, under which perithecia were produced at all light qualities tested. High moisture levels favor pycnidium formation, whereas dry conditions accelerate development of perithecia.

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Field samples of chrysanthemum (*Chrysanthemum morifolium* [Ramat.] Hemsl.) tissue infected by *Mycosphaerella ligulicola* Baker, Dimock, & Davis usually bear numerous pycnidia of the asexual stage (*Ascochyta chrysanthemi* Stevens). Less frequently, samples are found that predominantly bear perithecia or no reproductive structures. In most cases, such isolates produce abundant pycnidia when grown on potato-dextrose agar under fluorescent lights in the laboratory. Since environment appeared to be a decisive factor regulating reproductive development of *M. ligulicola* in the field, extensive controlled environment studies were made to determine the significant variables in the reproductive process.

Some 40 years elapsed between the first report of "ray blight" of chrysanthemum (12) and discovery of the sexual stage of the pathogen (1). Studies on reproduction of this fungus include observations on development of pycnidia and perithecia (1, 2), and some extensive investigations of pycnidium and conidiospore development (4, 5, 7). These reports indicate that the optimum temperature for mycelial growth is in the 23- to 24-C range. Both pycnidia and perithecia were formed on dry stems held at relative humidities from 6 to 98% in daylight, but only pycnidia were formed in darkness at these same relative humidity values (2). Pycnidium formation in vitro was erratic, some isolates sporulating readily, others only when exposed to daylight (1). Continuous white and near-ultraviolet (UV) light had little effect on pycnidium formation, but depressed the size and septation of conidia when compared to dark-grown cultures (5). Conidium size and per cent septation decreased as temperature increased, but spore number increased concurrently with temperature so that the volume of conidia produced remained constant over a wide temperature range (5).

MATERIALS AND METHODS.—Fresh chrysanthemum stem and leaf tissue (cultivar Fred Shoesmith), either whole or shredded, was used as a natural substrate for fungus growth. Except for the test relating osmotic potential (OP) to reproductive development, all chrysanthemum tissue was soaked in two changes of a 0.5 M NaCl solution for 12 hr to allow osmotic equilibrium to occur. The OP of 0.5 M NaCl is 20 atm (11). The OP of juice expressed from treated tissue was 15 to 18 atm as determined by water loss of leaf discs placed in the expressate, and in mannitol solutions of known OP. Tissue was inoculated by addition of a suspension of conidia in 0.5 M NaCl. The inoculated tissue was placed in sealed 10-cm-diam preparation dishes held at the desired conditions in controlled environment facilities. Though this technique was not aseptic, contamination was rare. For certain in vitro tests, potato-dextrose agar (PDA) was used as the substrate.

Unless otherwise stated for specific experiments, temperature was held at 20 ± 0.5 C, and lights were a fluorescent-incandescent combination with a 14-hr photoperiod, radiation intensity of 1,750 $\mu w/cm^2$, and an illumination level of 2,300 ft-c at the substrate surface. Light quality was varied by the placing of different filter materials consisting of red, blue, or green cellophane or white bond paper over cultures of the fungus. Variables studied were temperature, moisture, and light. Temperatures were recorded during the temperature experiment by thermocouple probes placed both in the plant tissue and in the ambient air of the chamber. Ambient chamber temperatures were programmed to drop ca. 2 C when the chamber lights

TABLE 1. Growth rate on potato-dextrose agar and time required for pycnidium development of *Mycosphaerella ligulicola* isolate mA_2 (an ascospore progeny of an isolate found in field infections in California) at nine different temperatures in in darkness

	Temperature (C)								
	3	6	12	18	21	24	27	30	33
Growth rate[a] mm/day	0	1.2	2.8	4.4	5.3	6.0	6.1	0.5	0
Days to pycnidia			13	9	7	5	3		

[a]Average of six cultures/temperature.

were on to offset radiative heating of the substrate, thereby maintaining a constant substrate temperature. Radiant flux densities (irradiance levels) were measured with a YSI-Kettering radiometer Model 65 fitted with a collector cone. Illumination levels were measured with a Weston foot-candle meter (target with a viscor filter and cosine correction), and light quality measurements were made with an ISCO spectroradiometer Model SR.

RESULTS.—*Temperature.*—Rates of growth and pycnidium formation were studied in vitro with isolate mA_2, an isolate not requiring light stimulation for sporulation, in total darkness. Growth rate tubes of PDA were seeded with 4-mm-diam mycelial discs cut from dark-grown colonies in culture plates. Six cultures were placed in each of nine unlighted incubators ranging in temperature from 3 to 33 C with a control accuracy of ± 1 C. Readings of radial growth and pycnidium development were made every other day for 15 days. Pycnidium development and radial extension followed essentially the same pattern (Table 1). The optimum temperature for both processes was near 26 C. Growth at 30 C was extremely limited, and no pycnidia were produced. No growth occured at 33 C.

In further tests using chrysanthemum tissue as a natural substrate, the cardinal temperatures for development of pycnidia and perithecia were found to differ significantly. Ten cultures were placed in each of five lighted growth chambers ranging from 10 to 30 C. The rates of development for both structures as functions of temperature are depicted in Fig. 1. The different temperature requirements for each stage are clearly seen. The temperature/pycnidium-development curve was similar to that for mycelium extension; i.e., maximum 30 C, and optimum ca. 26 C. Mature pycnidia were present 3 days from inoculation at 25 C, whereas 5 days were required at 20 C, and 10 days at 10 C. The optimum for perithecium development was 20 C, with no perithecium production at 25 C. Seven days were required from time of inoculation to perithecium maturation at 20 C; 25 days at 10 C.

A compounding effect is that of temperature on conidium number, size, and septation. Conidium size and the per cent of septate conidia decrease with temperature (Fig. 2, 3), whereas the number of conidia produced increases with temperature. This was plainly visible in our tests, although counts were not made. The net result, according to Blakeman & Hadley (5), is a constant total volume of conidia produced over a wide temperature range, with small numbers of large conidia produced at low temperatures, and large numbers of small conidia at high temperatures. This is strongly supported by our observations.

A third test of reproductive development was made at temperatures near optimum for production of perithecia. Isolate mA_2 was seeded on chrysanthemum tissue as before and placed in lighted growth chambers at 18, 20, 22, and 24 C. In addition, three fluctuating temperature treatments were included, with daily sinusoidal variations of 14 to 24 C, 20 to 32 C, and 20 to 40 C. Mean temperatures for each of the fluctuating temperature treatments were, respectively, 19, 26, and 30 C. Readings taken at 7 and 10 days from inoculation are recorded in Table 2. After 7 days at 18 C, asci were present; at 20 C, asci were well developed but no signs of cleavage were visible; at 22 C, asci were formed and immature ascospores were delimited from the cytoplasm. No evidence of perithecium development was visible after 10 days at 24 C. Conidium size and septation followed the same pattern as in Fig. 2.

Under the 14- to 24- and 20- to 32-C fluctuating temperature regimes, different sizes of conidia were present and the number of septa varied. Under the sinusoidal fluctuation of 20 to 40 C, no reproductive development took place; over half of each diurnal period was at a temperature higher than the maximum for vegetative growth of *M. ligulicola*, and all cultures were contaminated by numerous fungi. Perithecia were formed only under the 14- to 24-C fluctuation. In the 20- to 32-C regime, the temperature was above the maximum for perithecium development for more than 16 hr/day.

Light.—Two tests of reproductive development under different light regimes were conducted, one relating light quality and length of daily photoperiod to pycnidium formation on PDA, the other relating light quality at two intensity levels to development of pycnidia and perithecia on chrysanthemum tissue.

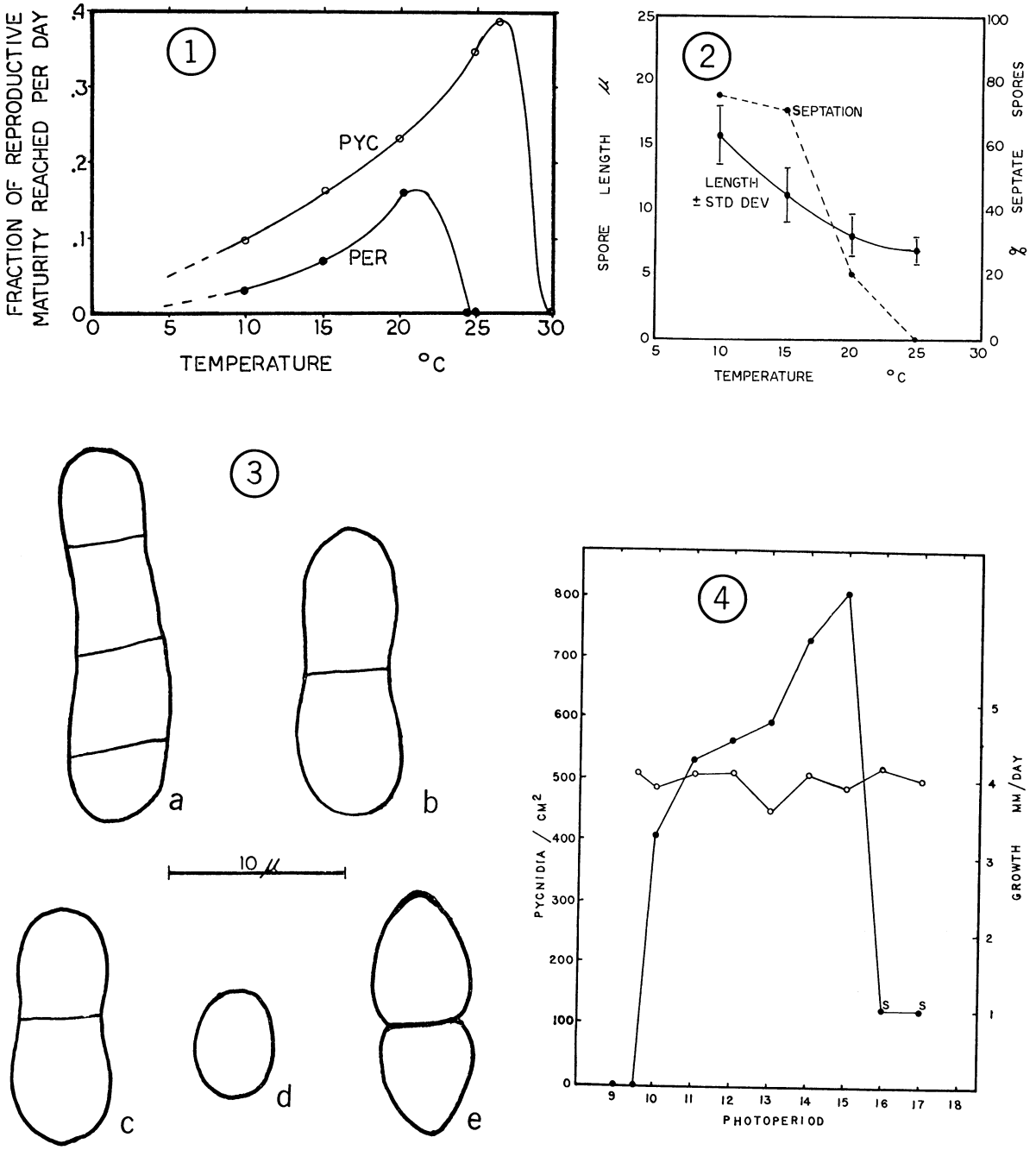

Fig. 1-4. **1)** Rates of development of pycnidia (PYC) and perithecia (PER) of *Mycosphaerella ligulicola* as functions of temperature. The ordinate is the reciprocal of the time in days to reproductive maturity. **2)** *Mycosphaerella ligulicola* conidium length and per cent septate conidia produced as functions of temperature. **3)** Spores produced by *Mycosphaerella ligulicola*. a, b = Conidia produced at 10 C, most are 2-celled as in (b), although some may be 2- or 3-septate as in (a). c = Two-celled conidium produced at 18 C; note smaller size. d = Unicellular conidium produced at 25 C. e = Ascospore. **4)** *Mycosphaerella ligulicola* pycnidia produced per cm^2 (solid circles) and rate of linear extension (open circles) of mycelium for isolate O-34 grown at daily photoperiods of 9 to 17 hr under a light bank including ultraviolet lights; s = sterile pycnidia.

Effects of light on pycnidium formation were studied by placing PDA cultures of light-sensitive isolate O-34, 41 cm below a light bank consisting of 2 40-w BLB fluorescent tubes, 2 40-w cool-white fluorescent tubes, and 4 100-w incandescent lamps. Light quality was varied by placement of filter materials having different wavelength cutoff points over the cultures. Light intensity measurements were not taken. Pycnidia were formed only under Pyrex glass, which has a wavelength cutoff of 280 nm below which transmission does not occur. Pycnidia were not formed under Mylar, Plexiglas, or window glass, which have wavelength cutoffs of 400, 340 and 310 nm, respectively.

Cultures were subjected to photoperiods ranging from 9 to 17 hr daily. Pycnidium densities were determined after 7 days from the average of four counts of random areas of 4 mm^2 on the agar surface. Results (Fig. 4) indicate that pycnidia were formed at photoperiods of 10 to 17 hr; no pycnidia were formed at photoperiods of 9 or 9.5 hr. Pycnidia produced at photoperiods greater than 15 hr contained no conidia. The rate of linear extension of mycelium remained constant over the range of photoperiods tested.

Reproductive development on shredded chrysanthemum tissue was studied under four light quality regimes at two intensities and in total darkness. Inoculated tissue was held 10 days under four filter materials at various distances from the incandescent-fluorescent light banks of a growth chamber maintained at 20 C. By adjusting the distance of the cultures from the light source two irradiance levels at the agar surface were established, 425 to 500 $\mu w/cm^2$ and 1,400 to 1,500 $\mu w/cm^2$. Corresponding illumination levels were approximately 700 and 2,500 ft-c. Spectral energy distributions were measured directly in the 380- to 750-nm wavelength range (Fig. 5) and estimated in the near UV and infrared from transmittances of the various filter materials as measured in a Beckman DB spectrophotometer (Table 3).

Isolates LI-2 and A-2 produced perithecia and pycnidia under blue or green filters at both intensities (Table 4). Cultures under red or white filters produced both structures at high intensities, but only pycnidia in the low intensity range. Dark-grown cultures of LI-2 exhibited sparse pycnidium development, whereas isolate A-2 developed no reproductive structures in the dark. Development of A-2 under the white filter was unique, high numbers of sterile pycnidia being produced at low intensity, whereas perithecia produced at the high intensity often reverted to pycnidia; i.e., the perithecium neck widened and conidia were produced within this widened neck (Fig. 6-B, C). Conidial cirri issuing from these structures contained occasional ascospores. A third isolate, mA_2, produced both pycnidia and perithecia with equal facility in all treatments, including total darkness. These data indicate that significant variations in light response exist among isolates of *M. ligulicola*.

A subsequent test of all available cultures of *M. ligulicola* was made to ascertain the extent of the variation in light response. Ten cultures, known to

TABLE 2. Reproductive development of *Mycosphaerella ligulicola* under near-optimal and fluctuating temperature regimes

Temp regime (C)	Perithecium maturity[a]		Conidia[b]		
	7 days	10 days	Pycnidia	Size	Septa
18	2	4	+[c]	Med +	Many
20	3	4	+	Med	Many
22	3+	4	+	Small +	Few
24	0	0	+	Small	None
14-24	2	4	+	Mixed	Mixed
20-32	0	0	+	Mixed	Few
20-40	0	0	–	None	

[a]Perithecium maturity ratings: 0 = no sign of perithecium development; 1 = initials present, neck not formed; 2 = perithecial neck formed, no asci present; 3 = asci present, no ascospores; 4 = mature ascospores present.

[b]Size: majority of conidia produced are: small, ⩽ 8 μ; medium, 8 to 12 μ; or mixed, small and large conidia present together. Septation: many = majority of conidia septate; few = majority of conidia not septate; none = no conidia septate; mixed = septate and nonseptate conidia present in approximately equal numbers.

[c]+ = present; – = absent.

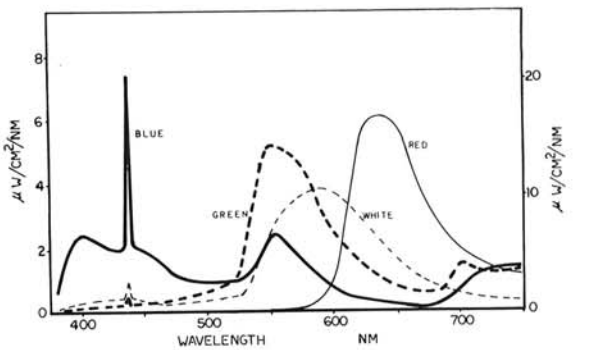

Fig. 5. Spectral energy distributions under four filter materials at two over-all irradiance levels: left ordinate 450 $\mu w/cm^2$; right ordinate 1,500 $\mu w/cm^2$:

Wavelength (nm)	% Transmittance				
	Blue	White	Red	Green	Plexiglass
950	66	0.5	20	41	91
900	66	0.7	21	40	90
850	65	0.5	20	40	90
800	85	0.5	20	40	91
750	62	1.0	21	39	91
700	30	1.0	20	29	91
650	1	10	18	7	90
400	52	8	6	5	80
350	8	0	15	10	75
300	0	0	0	10	45

TABLE 3. Infrared and ultraviolet transmittance of the filter materials used in the light quality experiment and of the plexiglass light barrier separating the light bank from the growth chamber

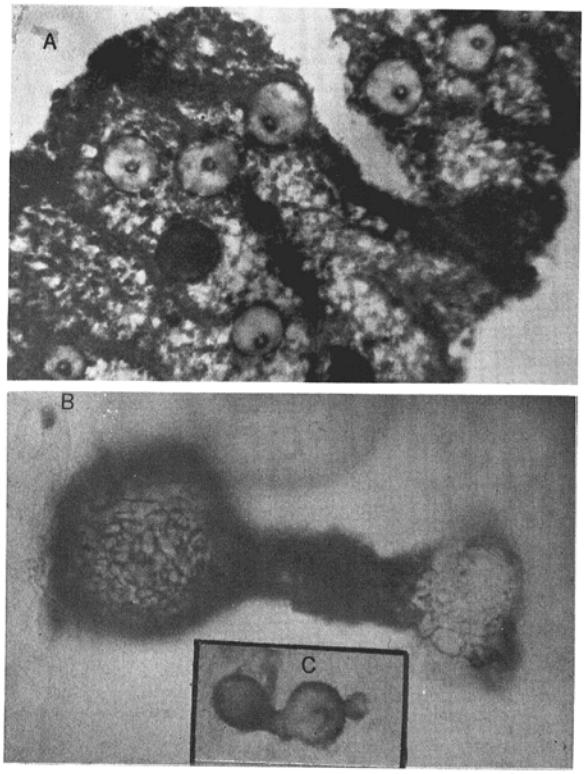

Fig. 6. A) Normal *Mycosphaerella ligulicola* pycnidia (light structures) and perithecia (dark structures) on chrysanthemum leaf tissue. Mean diameters are 150 μ. B, C) Perithecia of single ascospore culture A-2 produced under the white filter material at high light intensity (2,500 ft-c; 1,400 to 1,500 $\mu w/cm^2$). The perithecium neck has widened and has become continuous with a pycnidial structure.

produce perithecia, were grown on PDA in incubators at 20 C and kept in total darkness. The results indicate that *M. ligulicola* may contain at least two light response groups. One group requires light for either sexual or asexual reproduction, the other group has no light requirement. On PDA, light-insensitive cultures grown in the dark produce pycnidia evenly distributed over the agar surface, although both light-sensitive and -insensitive types produce pycnidia in well-defined annular rings when exposed to a daily photoperiod of 14 hr.

Moisture.—In many cases it was noted that cultures of *M. ligulicola* growing on shredded chrysanthemum tissue produced perithecia predominantly in the more elevated or drier portions of the medium. Tissue that was extremely moist or had not been soaked in 0.5 M NaCl solution usually yielded large numbers of pycnidia when seeded with *M. ligulicola*. Several tests were initiated to determine the relationship of substrate moisture content and OP to reproductive development. In the first test, chrysanthemum leaf and stem tissue was soaked overnight in two changes of 5-NaCl solutions. Osmotic potential of the solutions used were 0, 5, 10, 15, and 20 atm. Four replicate cultures of each OP treatment were seeded with a conidium suspension of isolate mA_2 in a NaCl solution of equal OP. After 10 days, counts were made of the relative numbers of perithecia and pycnidia in each treatment. Each count was based on ca. 100 structures taken from four randomly selected microscope fields. The ratios of perithecia to pycnidia from each replicate are plotted on a log scale against OP in Fig. 7. Results vary from a ratio of 12 pycnidia to one perithecium for tissue soaked in distilled water, to 10 perithecia to one pycnidium for tissue soaked in 20 atm NaCl. Tissue moisture contents were 4.1 ± 0.5 parts water/part dry weight of tissue.

A second test compared water content of tissue to rate of perithecium maturation in shredded tissue of constant OP. The tissue was soaked in a 20-atm NaCl solution until osmotic equilibrium was achieved, and then pressed to remove water. Osmotic potential of the expressate from all treatments was 18 atm. Moisture content was determined according to the formula

(fresh weight − dry weight)/ dry weight

expressed as a ratio. Three moisture levels were tested: 8:1, 5:1, and 2:1 (wet, moist, and dry to the touch, respectively). Readings of perithecium maturation were made 7, 10, 15, and 17 days after inoculation. Perithecia produced in tissue with a

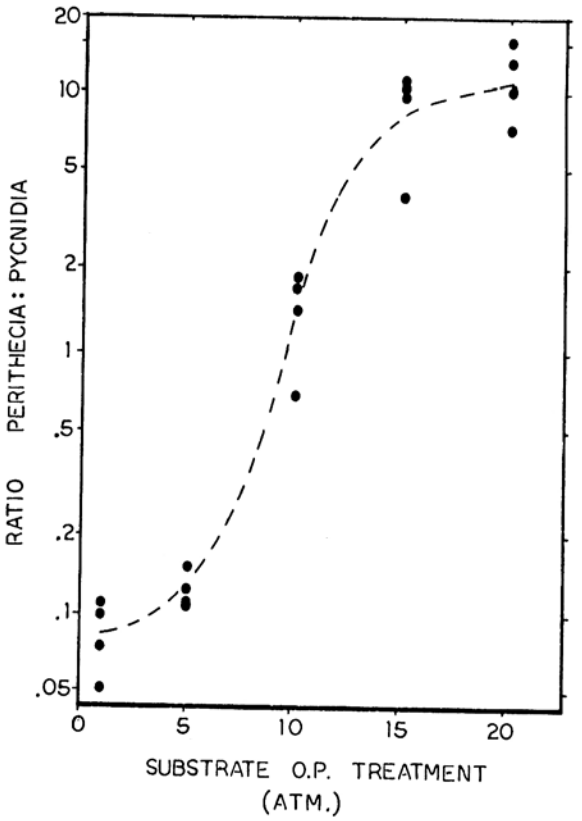

Fig. 7. Ratios of perithecia to pycnidia of *Mycosphaerella ligulicola* grown on chrysanthemum tissue soaked in NaCl solutions ranging from 0 to 20 atm in osmotic potential (OP).

moisture content of 2:1 were mature within 7 days, and tissue with a moisture level of 5:1 produced mature perithecia within 10 days. Tissue at an 8:1 moisture content had only a small number of mature perithecia 17 days after inoculation. Perthecia produced under the 8:1 treatment did not explosively discharge their ascospores, but the ascospores oozed out forming a gelatinous mass around the neck of the perithecium.

DISCUSSION.–The different temperature optima for the development of pycnidia and perithecia of *M. ligulicola* may explain the rarity of the perfect stage in nature. Temperatures optimal for pycnidium development may totally suppress perithecium formation. Furthermore, pycnidia are formed much more rapidly than are perithecia, even at temperatures favorable for perithecium formation. A lesion must be a minimum of 7 days old before perithecia may be present, whereas pycnidia may be produced within 3 days at the respective optimum temperatures. If a rigorous field control program is practiced, disease foci in which pycnidia have been produced may be detected and rogued out before perithecia develop and discharge airborne ascospores.

The effect of temperature on conidium morphology has implications both to disease development and to the taxonomic relationships of certain described pathogens of chrysanthemum. Blakeman & Fraser (4) reported that conidia produced at 26 C required a longer period for germination than did conidia produced at 15 C. The 15-C conidia were found to germinate at relative humidity values ranging from 97 to 100%; the 26-C conidia required free water for germination. They also reported a lower level of infection of leaf discs by conidia produced at 26 C than by those produced at 15 C. They concluded that the potential advantage of producing larger numbers of spores at higher temperatures would be realized only under optimum conditions for dispersal and infection.

The production of only one-celled conidia at temperatures greater than 24 C could lead to the misidentification of *M. ligulicola* as a species of *Phoma* or *Phyllosticta*. Field isolates of *M. ligulicola* grown on agar media under fluorescent lights often produce only one-celled conidia when held at laboratory temperatures. For this reason, care should be exercised in making identifications of pycnidial fungi isolated from chrysanthemum.

The effect of light on reproductive development of *M. ligulicola* is interesting from the biological point of view, but no significant relationship to development under field conditions is seen at this time. The development of pycnidia is enhanced by near-UV light (280 to 380 nm) only in those isolates that are intrinsically sensitive to light stimulation. Blakeman & Hadley (5) reported a 50% decrease in conidium production by *M. ligulicola* (dark sporulating strain) grown under continuous UV irradiation when compared to cultures grown in darkness, but not total

TABLE 4. Light quality versus reproductive development by three isolates of *Mycosphaerella ligulicola* grown on chrysanthemum tissue under nine light regimes

		Low intensity				High intensity			
		Conidia		Density, no./cm²		Conidia		Density, no./cm²	
Isolate	Filter color	Size (μ)	Septation	Pycnidia	Perithecia	Size (μ)	Septation	Pycnidia	Perithecia
LI-2[a]	White	10	+[f]	320	0	10	–	35	495
	Blue	10	–	10	540	10	–	45	480
	Green	10	–	85	475	10	–	20	510
	Red	12	+	590	0	10	–	10	615
	Dark	16	+	25	0				
A-2[b]	White	0		825[d]	0	10	–	125[e]	440[e]
	Blue	10	–	110	690	10	–	10	600
	Green	10	–	14	590	10	–	10	540
	Red	12	+	575	0	10	–	25	740
	Dark	0		0	0				
mA_2[c]	White	10	+	325	140				
	Blue	10	–	240	250				
	Green	10	–	310	190				
	Red	12	+	260	275				
	Dark	16	+	350	125				

[a]Isolated from a field infection in New York.
[b]Single ascospore progeny of isolate LI-2.
[c]Ascospore progeny of an isolate from a field infection in California.
[d]Pycnidia sterile, no conidia present.
[e]Some perithecia reverting to pycnidia.
[f]+ = septation; – = no septation.

inhibition as seen for isolate O-34.

It has not been ascertained whether the effect of photoperiod on pycnidium development is a true photoperiod response, or simply reflects a light-dosage requirement for the initiation of pycnidia. Blakeman & Hadley (5) reported that a 12-hour photoperiod had no inhibitory effect on sporulation. The development of sterile pycnidia at photoperiods greater than 15 hr is a phenomenon that appears occasionally among isolates of *M. ligulicola*. Sterile pycnidia were produced by isolate A-2 on chrysanthemum tissue under the white filter material at a low irradiation intensity level. Sterile pycnidia also have been observed from time to time on media inoculated with light-sensitive isolates and grown in darkness or under low levels of illumination. Hadley & Blakeman (7) reported that sterile pycnidia "invested with vegetative hyphae" were produced in darkness on media with an excess of carbon source or a low nitrogen level.

No conclusions are drawn with regard to the effect of light quality on perithecium formation. However, comparisons of the spectra involved indicate a possible interaction in the red-far red portion of the spectrum for those isolates responsive to light stimulation for development of pycnidia and perithecia. The spectra of the blue and green filter materials (which supported perithecium formation) have a higher proportion of light in the far red (700 to 750 nm) portion of the spectrum than in the near red (650 to 700 nm); i.e., 8.5 and 2.5 times, respectively, as opposed to 0.36 and 0.3 times for the red and white filters (Fig. 5). The existence of a red-far red interaction in fungi is not unfounded, in that Brooke (6) reported ascospore discharge by *Venturia inaequalis* to be stimulated by far red light, but inhibited by near red light. The fact that this effect is lost at higher light intensities is not surprising, since the red-far red phytochrome reaction in higher plants may be masked at high light intensities (13). A near-UV requirement for perithecium formation is precluded, since the red filter which did not support perithecium development at low light intensity passed more UV than did the blue filter which did support perithecium development (Table 4). Also, extensive pycnidium production occurred under the red and white filter materials; such pycnidium production appeared to require near-UV, witness isolate O-34. The fact that the white filter permitted little UV to pass may be responsible for the production of sterile pycnidia by isolate A-2 when grown under this filter.

The sensitivity or insensitivity of isolates to light appears to be carried through to other phases of the life cycle, and is manifest not only in reproductive development, but in ascospore discharge as well. Studies by McCoy & Dimock (10) indicated that ascospore discharge by certain isolates of *M. ligulicola* was inhibited by light (i.e., occurred only in darkness), whereas ascospore discharge by another isolate occurred in both light and dark. Isolates in which ascospore discharge is inhibited by light also failed to produce reproductive structures in continuous darkness. The isolate discharging ascospores in either light or dark also developed reproductive structures in both alternating light and dark and in continuous darkness.

A major conclusion drawn from the light studies is that there is variation in light response existing among isolates of *M. ligulicola*. This is not restricted to a few isolates, but has been noted in the previous literature on *M. ligulicola*. Baker et al. (1) reported that some isolates produced pycnidia readily in culture in the laboratory; others failed to produce pycnidia in culture but did so readily on inoculated blossoms outdoors. J. P. Blakeman (*personal communication*) stated that all his cultures have been dark-sporulating types. Variation in light response among isolates is not unique to *M. ligulicola,* but has been reported for *Leptosphaerulina arachidicola* and *L. briosiana* (9), for *Ascochyta pisi* (8), for *Botrytis squamosa* (3), and for *B. cinerea* (R. E. Hite, *personal communication*). Obviously the extrapolation of light response data to field conditions should be made with caution, possibly not without first making a survey of the light response groups in the fungus population.

Mycosphaerella ligulicola has long been noted to produce reproductive structures under desiccatory conditions. Baker et al. (2) noted that perithecia had been produced on herbarium specimens that had been placed under arid storage conditions. Blakeman (*personal communication*) observed perithecium formation on dilute PDA after a period of 6 weeks, especially when the relative humidity in the culture plates was kept low. These reports are in keeping with the results obtained in this study. Low moisture availability appears to swing reproductive development towards perithecium production, whereas mostly pycnidia are produced in the absence of moisture stress, providing that other environmental factors are favorable.

Extrapolated to the field, this indicates that pycnidia would most probably be produced during wet spells when splash dispersal is most effective. Perithecia produced under high moisture conditions are slow to mature and, if maintained under moist conditions, may not violently discharge their ascospores into the air. A similar, though inverse, seasonal variation in perithecium production has been reported for *Mycosphaerella musicola,* in which perithecium production occurs during the tropical rainy season, but not during the dry season (R. H. Stover, *unpublished data*).

The observed field development of Mycosphaerella blight in New York indicates that the disease is confined to localized foci during the warm summer months. During this time, while the crop is vegetative, spread is primarily by splash dispersal of conidia. As cooler weather develops, perithecia are formed and airborne dispersal of ascospore inoculum begins as the crop comes into flower, thus initiating the destructive ray-blight phase of the disease. The two pleomorphic stages of *M. ligulicola* serve important purposes in the disease cycle; pycnidia serve to enlarge established disease foci, and perithecia are responsible for the establishment of new foci and for the spread of inoculum to the uppermost floral tissues of the plant.

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