OVERWINTERING OF UROMYCES STRIATUS SCHROET. AND SOME EFFECTS OF TEMPERATURE, PHOTOPERIOD, MOISTURE AND LEAF AGE ON ALFALFA RUST DEVELOPMENT

by

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TABLE OF CONTENTS

PART I. OVERWINTERING OF *UROMYCES STRIATUS* UREDIOSPORES IN ALFALFA HAY

Rust of alfalfa (*Medicago sativa* L.), caused by *Uromyces striatus* Schroet., is widespread in the temperate zones of the world (2). In the U.S. *U. striatus* reportedly persists in alfalfa leaf tissue in the milder climates and spreads northward by means of urediospores during the summer (3). The disease usually is first reported in Kansas in July or August (11, 12, 13, 14) and builds up until the alfalfa fall growth is killed by low temperatures. In Canada, the mycelium is perennial in the aecial host *Euphorbia cyparissias* L. (8). This alternate host occurs in approximately the northeast quarter of the U.S. (1, 5) but the aecial stage has not been reported there. Leppik (6) reported that *U. striatus* teliospores were rarely found on alfalfa in America. In Bulgaria, *U. striatus* overwinters mainly in the uredial stage on alfalfa stem and leaf debris (9). The alternate host plays only a minor role in infection there because aecia appear on the alternate host after infection is well established on alfalfa.

Some of the *U. striatus* urediospores that Maneval (7) collected November 11 in Missouri and stored in a cool room for six months germinated and infected alfalfa. Urediospores collected during March and April, however, did not germinate. Maneval's data suggest that *U. striatus* urediospores might overwinter in Kansas and I report here research initiated to determine this.

MATERIALS AND METHODS

Heavily rusted alfalfa shoots from fields near Manhattan, Kansas, were harvested on October 13 in 1977 and 1978, placed in burlap bags, and stored in an unheated building. At two-week intervals leaflets were collected from storage and from the field. From each sample at least 16 leaflets were washed in 50 ml distilled water to remove the spores and the suspension was then

passed through a tea strainer to remove plant debris. To determine spore germinability, drops of the suspension were pipetted onto glass slides on wet filter paper in petri dishes and incubated at 20 C in the dark. Six hr later 200 spores on each of three slides per sample were examined to determine the germination percentage. Germination percentages were often difficult to determine after 6 hr due to germ tube destruction by bacteria. To determine pathogenicity, a portion of the spore suspension from each sample was used to inoculate 60, 20-day-old Kanza alfalfa plants grown at 20 C and a 12-hr photoperiod. Following inoculation, the plants were placed at 20 C for 24 hr in a tight, darkened plastic box to maintain moisture. The plants then were transferred to 20 C with 8 klx of continuous fluorescent lighting and after 1 wk were observed daily to determine the latent period. Latent period was considered as the time between inoculation and first erumpent pustule.

RESULTS AND DISCUSSION

A low percentage of *U. striatus* urediospores remained viable overwinter on alfalfa shoots in storage but no viable ones were found in samples collected from the field during February or later in either 1978 or 1979 (Table 1). Viability of urediospores from the field and storage was about equal through December in 1977, but was considerably greater in the field in 1978 because urediospores were produced in the field well beyond the October 13 harvest date.

Field samples collected prior to January 1 included mostly leaves picked from standing shoots. However, leaves fell or blew off and were not available in later collections which included standing stems and leaves on the soil and often under snow. No urediospores collected from under the snow germinated although some had germ tubes when collected indicating that they had germinated, probably in moisture provided by the snow.

TABLE 1. Mean monthly germination percentages of *Uromyces striatus* urediospores from rusted alfalfa shoots collected from the field at indicated months and from shoots collected in the field on October 13, 1977 and 1978 and stored in an unheated building until tested

Month of sampling	1977-1978		1978-1979	
	Field	Storage	Field	Storage
Oct.	84.5	89.0	91.8	85.8
Nov.	71.2	82.9	91.1	75.7
Dec.	56.0	62.4	88.6	41.5
Jan.	8.1	25.4	--	--
Feb.	0.0	9.5	0.0	6.2
Mar.	0.0	4.4	0.0	7.8
Apr.	--	2.7	--	4.8
May	--	1.2	--	3.2
June	--	--	--	3.3
July	--	--	--	2.7

In July 1979, 2.7% of the urediospores from storage were still viable and at least some of them were pathogenic in the laboratory. However, the latent period increased as the storage period of urediospores used as inoculum increased. The mean latent period at 20 C during the months October to May 1977-78 was 9, 9, 10, 10, 11, 12, 14 and 14 days, respectively. In 1978-79, the mean latent period was 9 days through December, 10 days in February, 13 days in March and 14 days thereafter.

The cause of declining *U. striatus* urediospore germination in storage was not studied. Wynn et al (16) attributed loss in germinability of *Puccinia graminis* urediospores to their inability to metabolize available food sources such as lipids and carbohydrates because of a decline in enzyme levels and/or enzyme activity.

The decline in urediospore germinability with increased storage duration resulted in fewer pustules on plants inoculated to determine pathogenicity. Those pustule numbers were recorded only for the 1977-78 storage period. The mean number of pustules per leaflet on 20 of the most severely rusted leaflets from the 60 test plants were recorded when less than 25. The mean pustule number per leaflet was greater than 25 for October and November and was 14, 8, 4, 3, 2 and less than 1, respectively, for the months December through May. It has been shown with *Uromyces phaseoli* (15) and *Puccinia hordei* (10) that latent period increases as pustule density decreases. However, it is doubtful that the density of *U. striatus* pustules was sufficient, particularly after December, to have contributed noticeably to the increased latent period.

To determine pathogenicity in the field of urediospores that overwintered in storage, rusted shoots from storage were broken and scattered over alfalfa plants on several cloudy and rainy days from March through May 1979. Uredia were first observed May 5 on plants inoculated April 18. Rust buildup at those inoculated field sites was not followed closely but rust increased there

throughout the summer. Rust on the third and fourth hay cuts harvested August 2 and September 14 in plots adjacent to those inoculated areas was much more severe than in any of several other alfalfa fields observed in the area.

Contrary to Leppik's (6) observation that teliospores were rare on alfalfa in America, teliospores were observed in all samples from the field and storage and comprised 10-15% of the spores. They were observed only in pustules with urediospores; no separate telia were found. In the laboratory, teliospores appeared 20-25 days after inoculation on plants used in pathogenicity tests and were in about the same proportions as observed in field collections. Teliospore germination was not observed.

In the spring of both 1978 and 1979, alfalfa fields near Manhattan, Kansas, all upwind from inoculated field plot in 1979, were observed weekly for rust. The first rust pustules were observed on alfalfa July 17, 1978 and June 6, 1979. If primary inoculum is urediospores blown in from southern U.S. as Dickson (3) suggested, then rust would be expected in southern Kansas before these dates. First report of alfalfa rust in disease surveys in Kansas during those years were August 4, 1978 (13) and August 10, 1979 (14).

U. striatus apparently failed to overwinter in the field because otherwise viable urediospores germinated in rain or snow moisture in the absence of green host tissue. However, one cannot rule out the overwintering of *U. striatus* in Kansas during mild winters with abnormally low precipitation. My data suggest that if urediospores are protected from moisture such as in hay and on stored haying equipment, a low percentage can survive the winter in Kansas and serve as primary inoculum the following spring. The probability of that occurring is increased by recent changes in alfalfa management practices by some Kansas producers. Fall growth, formerly left to catch snow, is removed as a hay crop to remove alfalfa stems containing fall-laid eggs of the eastern strain of alfalfa weevil (*Hypera postica* Gyllenhal) (4).

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PART II. VIABILITY OF *UROMYCES STRIATUS* UREDIOSPORES DURING STORAGE AT -20 C

Longevity of fungus spores varies with species, type, and environmental stresses. Considerable interest has been expressed in longevity of rust urediospores because of their possible role in overwintering and initiating infection the following season. Cochrane (3) classified urediospores as having intermediate longevity compared with long-lived spores, such as chlamydospores of Ustilaginales, and short-lived spores, such as conidia of the powdery mildew fungi. Maneval (8) found that *Uromyces striatus* Schroet. urediospores germinated after 6 months storage in a cool room. Urediospores of *U. phaseoli* stored at -60 C for 670 days germinated 40% without hydration, but more than 70% germinated when hydrated 96 hr in a moist atmosphere before testing germination (11). Those stored at 23, -13, or -16 C were dead after 1-5 months. Other workers (5) found that some *U. phaseoli* urediospores stored at -20 C for two years were still viable, while those stored in a refrigerator at 2-5 C were dead after 3-4 month. Bailey (1) found 20% of *Puccinia helianthi* urediospores viable after 185 days at 8 C, while only 10% were viable when stored at 20-23 C. Doran (4) reported that only 50% of *Puccinia antirrhini* urediospores germinated after storage at 0 C for 7 days. *Puccinia graminis* urediospores hardened by holding 10 days at 0 to 1 C before storing at -29 to -40 C retained a slightly greater germination percentage than did non-hardened ones (10). Urediospores of *Phragmidium mucronatum* on leaves and in vials remained viable at -15 C only 56 days, while at 3-6 C and 25-50% relative humidity, they remained viable more than 300 days (3).

The purpose of this investigation was to determine the viability of *U. striatus* urediospores during storage at -20 C.

MATERIALS AND METHODS

Urediospores from one pustule were scraped from an alfalfa (*Medicago sativa* L.) leaflet into a small watch glass containing distilled water (7).

Healthy leaflets, inoculated by dipping into the spore suspension, were floated dorsal side up on a solution of 3% sucrose in sterilized tap water in petri dishes (6). These were placed at 20 C with a 12-hr photoperiod of 8 klx of fluorescent lighting. Fourteen days later the urediospores produced were collected and used to inoculate more excised leaves. The process was repeated several times to produce sufficient urediospores. Then urediospores were harvested from the excised leaves and collected in 1-ml ampules with a cyclone collector (2). The ampules were covered with Parafilm M, and stored at -20 C. Monthly 5 mg urediospores from storage were mixed in 50 ml distilled water and drops of the spore suspension were placed on microscopic slides on wet filter paper inside petri dishes. The germination percentage for a minimum of 200 urediospores from each of three replications was determined 6 hr later. To determine pathogenicity, a portion of the spore suspension was sprayed onto 20-day-old alfalfa seedlings. Following inoculation, the plants were placed in darkened boxes (to prevent drying) for 24 hr at 20 C and then were transferred to 20 C with continuous light until rust pustules appeared.

RESULTS AND DISCUSSION

Over 99% of fresh urediospores germinated by 6 hr. Viability in storage decreased to 93.8% after 1 month and declined steadily to 13.1% during 23 months (table 1). The loss of urediospore viability during storage was not determined. Wynn et al (13) attributed viability loss in *P. graminis* urediospores to their inability to utilize endogenous substrates due to a general decline in enzyme levels or enzyme activity.

The latent period requirements increased with storage time of urediospores used as inoculum. The latent period was 9 days with urediospores stored up to nine months and increased to 14 days as the storage period was increased to 23 months (Table 1). The decline in urediospore germination during the test period was reflected in a reduction in pustule numbers per leaf.

TABLE 1. Effect of storage period at -20 C on *Uromyces striatus* urediospore germinability and rust latent period

Storage period (Months)	Germination (%)	Latent period (Days)
1	93.8	9
2	90.6	9
3	91.6	9
4	87.9	9
5	85.4	9
6	86.6	9
7	81.3	9
8	80.4	9
9	74.6	10
10	70.7	10
11	67.8	10
12	69.2	11
13	62.3	11
14	61.6	11
15	59.2	12
16	38.0	12
17	24.2	13
18	17.2	13
19	16.8	13
20	16.9	13
21	15.2	14
22	14.1	14
23	13.1	14

Thus, the effective inoculum density decreased during the testing period and may have contributed to the increased latent period as reported for *U. phaseoli* (14) and *Puccinia* spp. (9, 12). Further progress on the problem, especially the relation between storage period of *U. striatus* urediospores and latent period, will require more knowledge of the processes involved in urediospore germination and the establishment of rust infection.

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PART III. SOME EFFECTS OF TEMPERATURE, PHOTOPERIOD, MOISTURE AND LEAF AGE ON ALFALFA RUST DEVELOPMENT

Rust of alfalfa (*Medicago sativa* L.), caused by *Uromyces striatus* Schroet. often is an important foliar disease in Kansas during July through November (28, 29, 30, 31). The various factors which influence the growth, development, and epidemiology of this rust have not been studied.

The minimum and maximum temperature limits for *Puccinia graminis* urediospore germination on wheat, barley, and oats are 2 and 31 C (9). The limits for *Puccinia coronata* on oats are 7 and 30 C (9). Ward (32) reported that *Puccinia dispersa* urediospores germinated best at 20 C, slightly at 27 C, and did not germinate at 30 C. The optimum temperature range for *P. coronata* urediospore germination is 16-25 C (15).

Studies to determine temperature effects on latent period have often yielded conflicting results. Teng and Close (27) reported that increasing temperature from 10 to 25 C decreased latent period of *Puccinia hordei* on barley from 12 to 5 days. Simkin and Wheeler (26) showed that increasing temperature from 5 to 25 C decreased *P. hordei* latent period from 60 to 6 days. However, Parlevliet (18), working with many barley cultivars, found no significant differences in latent period of *P. hordei* at four temperatures from 2 to 22 C. The latent period of *Puccinia recondita* was 2-3 days shorter at 19 C than at 15.5 C (6). Saari and Moore (23) noted that flecking of *P. coronata* occurred 2-3 days earlier at 29.5 C than at 18 C, but Kochman and Brown (11) reported that temperatures between 20 and 35 C had no significant effect on latent period of that pathogen.

Temperature effects on the sporulation period, i.e. the time between the beginning and cessation of spore production (4, 19), have been investigated for several rust fungi. Temperatures between 10 and 20 C had no significant

effect on the length of sporulation period of *P. hordei* on barley leaves (27). Under optimal conditions *P. recondita* sporulated for 55 days (13), *Puccinia polysora* for 18-20 days (3), and *P. coronata* for 30 days (5). Prabhu and Wallin (21) found that one rust pustule of *P. graminis* sporulated for 16 days at 24 C.

P. recondita urediospore production was threefold greater at 24 than at 15.5 C (6). *P. hordei* produced 188% more urediospores at 25 C than at 10 C (27). *P. graminis tritici* produced 415% more urediospores at 24 than at 13 C (21). *P. graminis avenae* spore production was 75% greater at 30 than at 20 C (11). The total weight of *P. hordei* urediospores collected in 9 days from an unspecified number of pustules was 166 mg at 10 C, 210 mg at 15 C, and 360 mg at 20 C (26). The maximum number of urediospores produced per pustule on any single day was 840 by *P. hordei* (27), 700 by *P. recondita* (13), and 700,000 by *Uromyces phaseoli* (34).

Uromyces phaseoli daily spore production per pustule was influenced by pustule density (34). It was 30,000 for 0.2-2 pustules per cm^2, 15,000 for 2-10 pustules, 4,000 for 10-20 pustules, 500 for 20-50 pustules, and only 200 spores for 50-150 pustules at 15 days after inoculation. Shaner et al (25) found that on all wheat cultivars tested, the pustule size of *P. recondita* decreased as pustule density increased. Increasing pustule density of *P. hordei* also resulted in decreased pustule size and urediospore number per pustule (27). Similar results were reported with *P. recondita* (11) and *U. phaseoli* (34).

The relation of environmental conditions to plant disease development frequently is based on meterological records that may not adequately represent the microclimate or environmental conditions near the plant. Yet the microclimate is very important in the establishment of a pathogen and in its subsequent buildup and spread (33). Further, each pathogen, and sometimes

each stage of pathogen development, may have its own environmental requirements (35).

The research reported here was initiated to obtain information on the effects of temperature, photoperiod, postinoculation moisture period, and leaf age on alfalfa rust development.

MATERIALS AND METHODS

Fungus isolate.—A single pustule isolate of *U. striatus* from the field was increased as previously described (1, 7). Fresh urediospores were produced as inoculum for each experiment. To do this alfalfa leaves were placed in petri dishes then atomized lightly with water and inoculated with dry stock urediospores discharged vertically in a turntable settling tower (16). The leaves were again atomized with water and the dishes were covered and placed in the dark at 20 C for 24 hr and then were transferred to petri dishes each containing 5-10 ml of 60 ppm benzimidazole solution (1). The dishes were placed at 20 C with a 12-hr photoperiod of 8 klx of fluorescent lighting. Fourteen days later, the urediospores were collected with a cyclone collector (2) and used as inoculum for an experiment.

Host plants.—Kanza alfalfa was seeded in 9.5 x 9.5 x 8 cm plastic pots containing a steam sterilized 3:1 sand-peat mixture. All the pots were placed at 20 C with a 12-hr photoperiod. Five days later, the seedlings were thinned to four per pot; one in each corner. The seedlings were irrigated with 10% Hoagland's solution except that 100% KNO_3 was used (8). Plants 20 days old were inoculated with fresh urediospores by using a turntable settling tower as described by Melching (16). After placing a pot on each of the 16 subturntables, 20 mg of dry urediospores were discharged upward at 5.62 kg/cm^2 (80 psi) into the center of the tower while the pots were rotating at 40 rpm and the main turntable was rotating at 20 rpm. The turntables continued to turn for 10 minutes after spore release.

Following inoculation, plants were placed in darkened mist cabinets at 20 C for 24 hr and then were transferred to specific experimental conditions. Each closed glass mist cabinet (60 x 35 x 40 cm) was placed inside a growth chamber and provided a distilled water mist (1 liter water per 24 hr) by a DeVilbiss No. 841 continuous flow nebulizer. This saturated the cabinet atmosphere and provided a film of water on the alfalfa leaves.

To determine urediospore germinability, drops of spore suspensions were placed on sterilized glass slides on a wet filter paper and incubated inside closed petri dishes. A minimum of 200 spores per treatment were examined 6 hr later to determine the germination percentage.

Spore production.—Pustules were counted every 48 hr (just before collecting the spores). Spores were removed from the leaves by vacuum (400 mm Hg) through a 4 mm inside diameter soft rubber hose as the hose end was gently rubbed across the leaves. The other end of that hose was attached to a 4 mm diameter glass tube which extended through the rubber stopper of a 2.5 x 20 cm test tube and to within 1 cm of the bottom. One end of another 4 mm diameter glass tube was extended barely through the stopper into the test tube and the other end was connected with a rubber hose to a vacuum pump. Twenty ml of a solution of 2% lithium chloride in methanol (10, 22, 24) was placed in each test tube. The spores were collected in the solution as the air was drawn through it.

A model B Coulter Counter (22) was used to count the number of spores per 0.5 ml from each treatment. Five readings per treatment were taken and the average was corrected by substracting the average of five background readings. Background readings represented the number of particles i.e. other spores, dust, hairs collected from healthy leaves.

RESULTS

Effect of temperature on urediospore germination.—To determine the temperature range for *U. striatus* urediospore germination. Drops of spore

suspensions were placed on glass slides at 5, 10, 15, 20, 25, and 30 C. Germination percentages were recorded 1, 2, 4, and 6 hr later. Germination percentages at 1 hr were 42, 39, and 8 at 15, 20, and 25 C, respectively. At 5 and 10 C germination was first observed at 2 hr (Fig. 1). However, by 6 hr over 99% of urediospores at 5, 10, 15, 20 and 25 C had germinated. No urediospores at 30 C germinated in 6 hr or when transferred to 20 C for another 4 hr.

To more closely determine temperature effects on urediospore germination, similar experiments were conducted at 12, 14, 16, 18 and 20 C and germination percentages were determined at 30 minute intervals for 4.5 hr. No germ tubes were observed at 30 minutes but 38.7, 38.6 and 37.9% of the urediospores germinated by 1 hr at 16, 20, and 18 C, respectively, and germination percentages at those temperatures, were not significantly different at any observation (Fig. 2). Germination at 12 and 14 C was first observed at 1.5 hr but by 4.5 hr over 99% of urediospores at all temperatures had germinated.

Effect of temperature and photoperiod on alfalfa rust development.— Following their inoculation, pots of 20-day-old alfalfa plants were transferred to darkened mist cabinets at 20 C for 24 hr and then were placed at photoperiods of 12, 14, 16, and 24 hr at 15, 20, 25, and 30 C. Each of the 16 treatments included two pots (8 seedlings, each with 3 expanded leaves at time of inoculation). This experiment was repeated twice with each experiment treated as a replication in the statistical analysis.

Latent period. The mean period from inoculation to appearance of first pustules was 169, 213, 269, and 310 hr at 25, 20, 15, and 30 C [LSD (P = 0.05) = 1.96], respectively (Fig. 3). Photoperiod had no significant effect on latent period at any of the temperatures tested.

Pustules per leaf. Pustules occurred on both sides of the alfalfa leaves but more than 50% were on the lower side. The number of sporulating

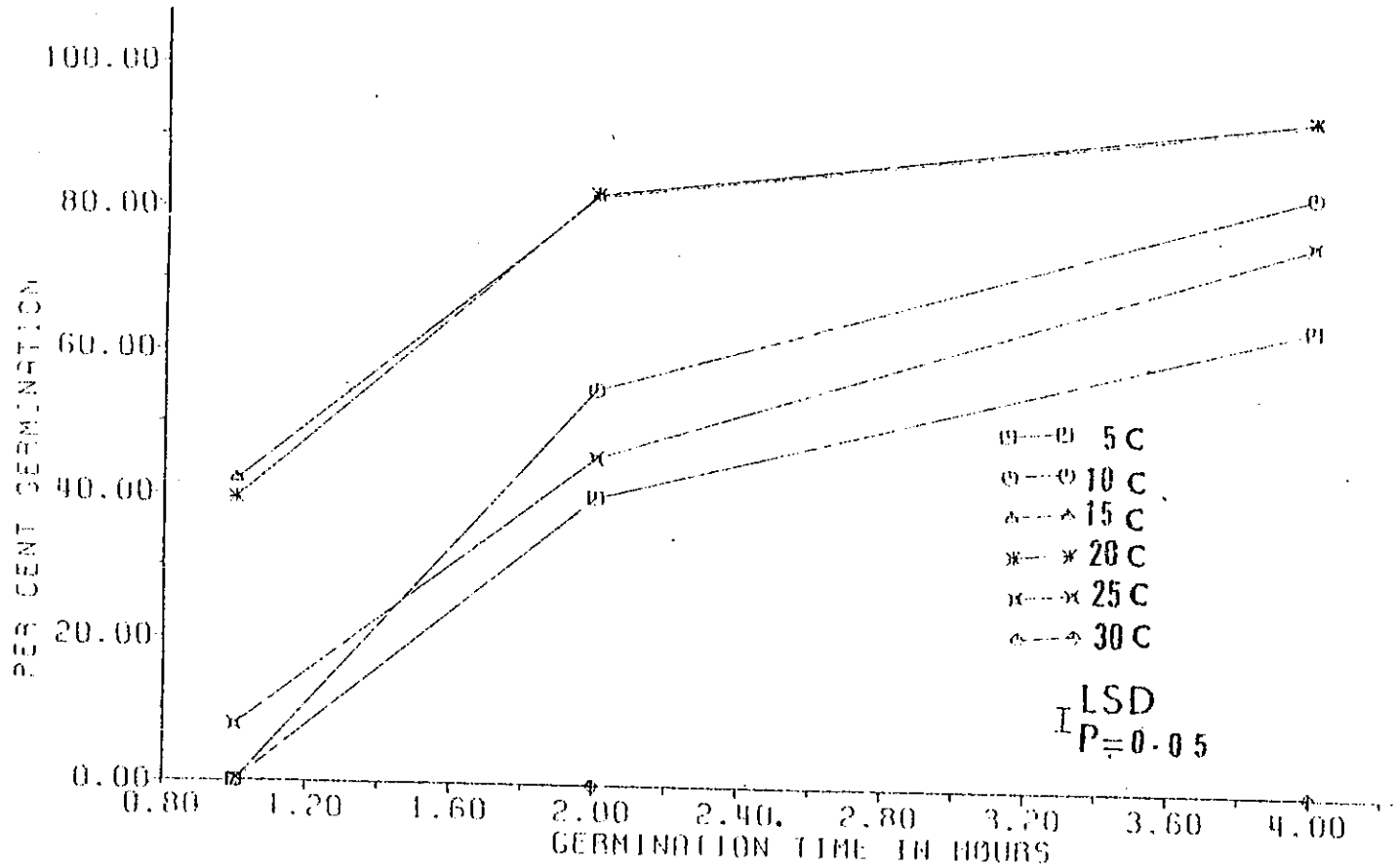

Fig. 1. Effect of temperature of the germination of *Uromyces striatus* urediospores.

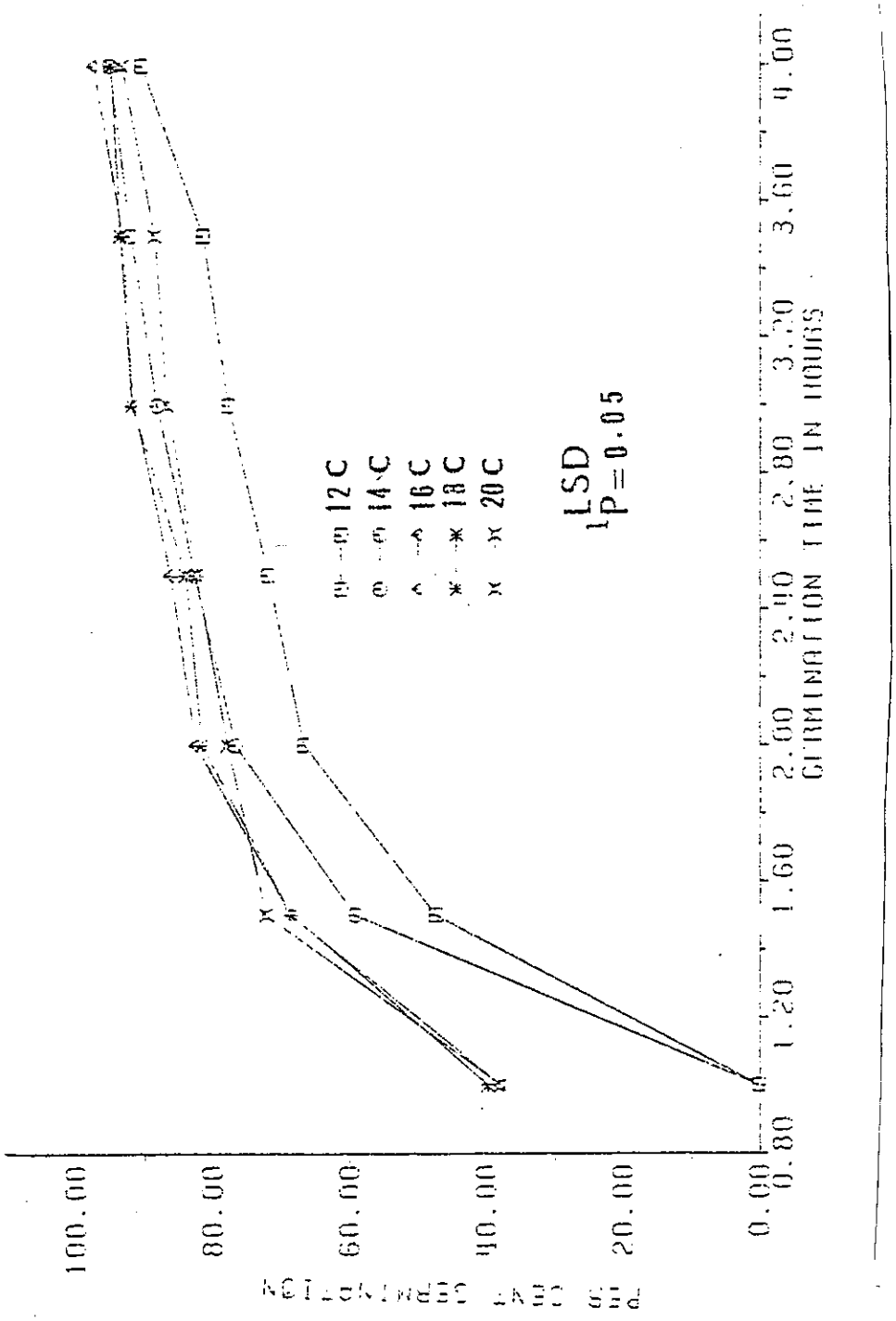

Fig. 2. Effect of temperature on the germination of *Uromyces striatus* urediospores.

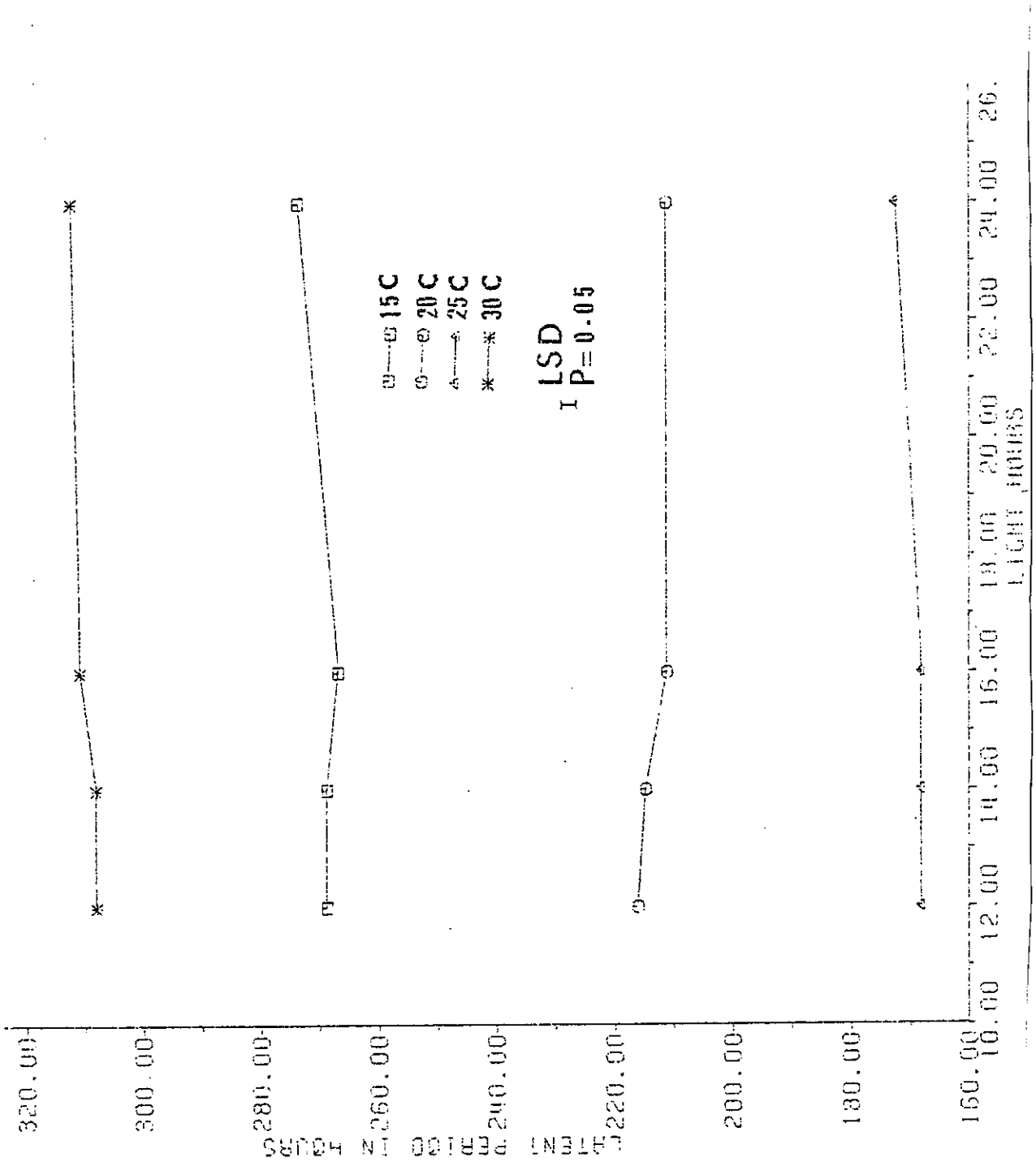

Fig. 3. Effect of temperatures and photoperiods on the latent period of *Uromyces striatus*.

pustules per leaf were counted every 2 days and just before harvesting the spores. Pustule numbers per leaf was affected by the temperature range more than by the photoperiod range tested and was significantly greater at 25 C than at any other temperature at all four photoperiods (Figs. 4-7). The greatest number of pustules per leaf at 25 C was 77, 76, 73, and 59 [LSD (P = 0.05) = 3.3] at photoperiods of 16, 14, 12, and 24 hr, respectively (Fig. 10). At those photoperiods (Figs. 4-7), pustules per leaf at 20, 15, and 30 C were 87-88, 84-71, and 41-30% as numerous as at 25 C. At every temperature tested, except 30 C, pustule numbers per leaf were greatest at the 16-hr photoperiod and decreased successively at photoperiods of 14, 12, and 24 hr (Figs. 8-11). At 30 C (Fig. 11), that order differed only by the mean pustule number at the 24-hr photoperiod being greater, although not significantly greater, than at the 12-hr photoperiod.

The peak number of sporulating pustules per leaf occurred 13, 17, 21, and 21 days after inoculation at 25, 20, 15, and 30 C, respectively, and was the same at photoperiods of 12, 14, and 16 hr (Figs. 4-6). The peak occurred 2 days later at each temperature at the 24-hr photoperiod (Fig. 7). Although infection by all spores occurred the same day, at 30 C and a 24-hr photoperiod (Fig. 7) some required 12 more days to produce pustules. Also some pustules produced spores for only 4-6 days while others sporulated for nearly 30 days. Near the end of the sporulation period a ring of 4-6 small secondary pustules developed around 0-2 primary pustules per leaf in all treatments. Secondary pustules were not included in the pustule counts.

The sporulation period was 34 days at all 9 combinations of 15, 20, and 25 C with photoperiods of 12, 14, and 16 hr (Figs. 4-6). It was 32 days at the 24-hr photoperiod (Fig. 7). At 30 C alfalfa plants lost vigor and 25-30 days after inoculation, the infected leaves wilted.

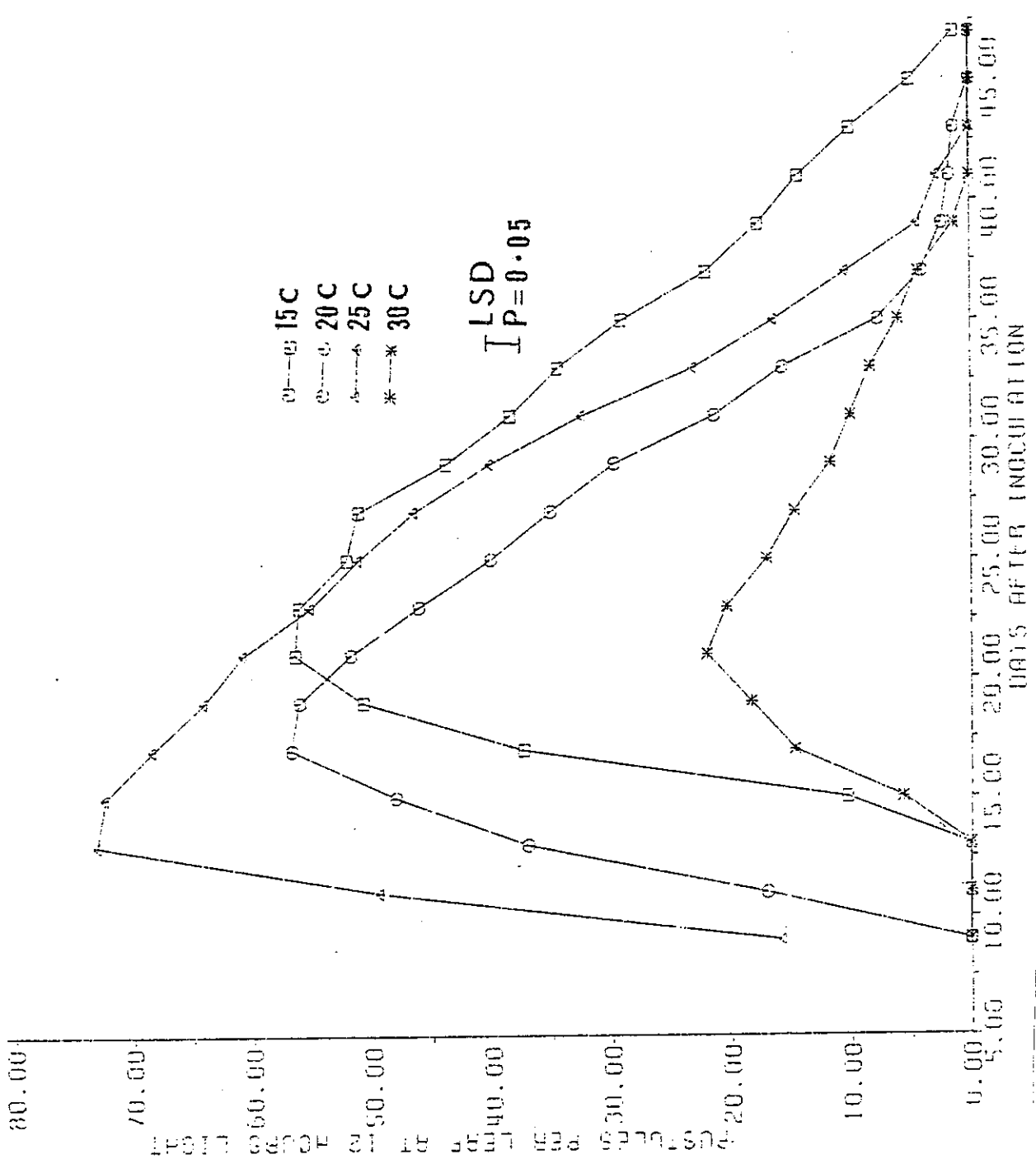

Fig. 4. Effect of four temperatures at a 12-hr photoperiod on the number of sporulating pustules of *Uromyces striatus* per alfalfa leaf.

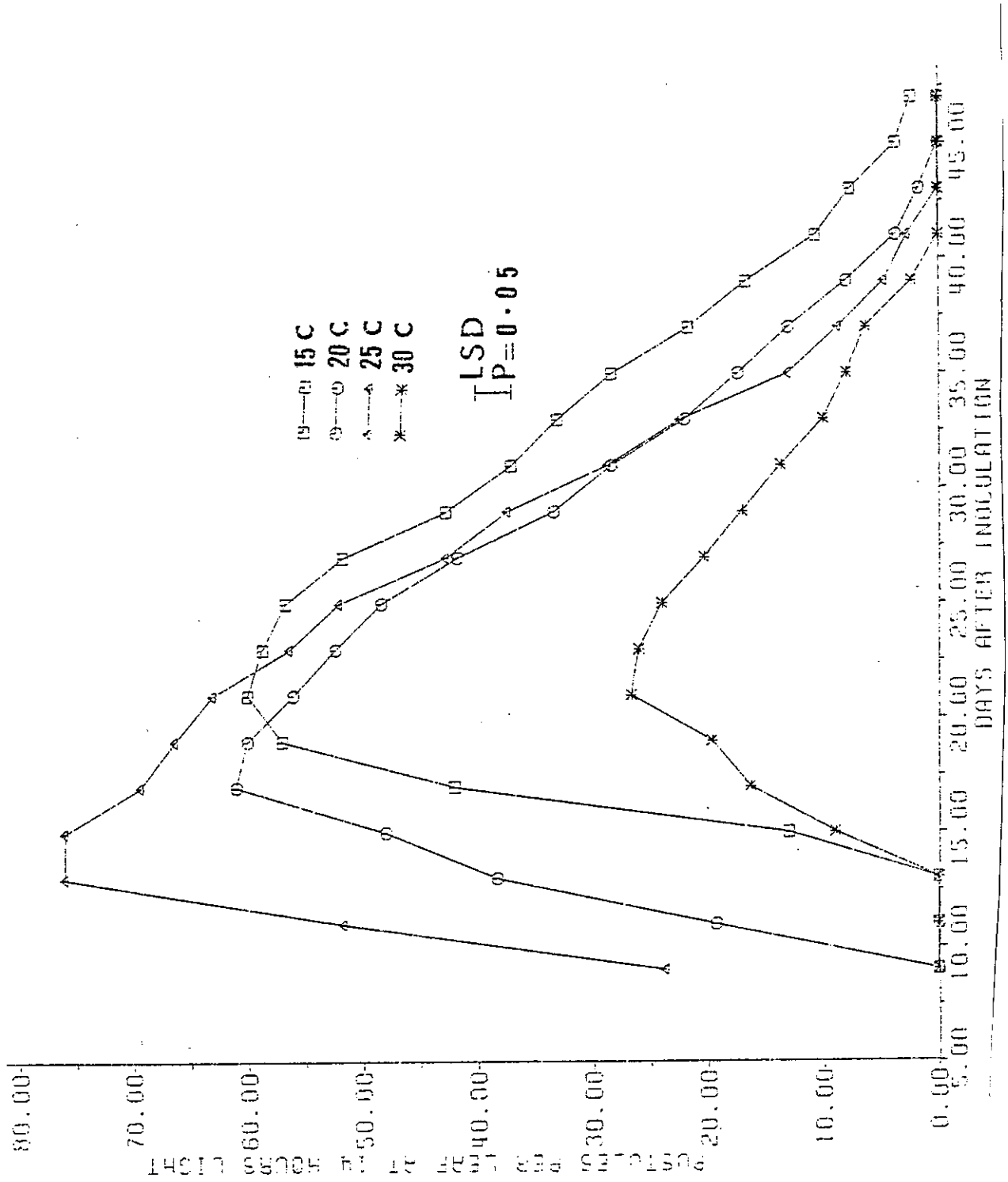

Fig. 5. Effect of four temperatures at a 14-hr photoperiod on the number of sporulating pustules of *Uromyces striatus* per alfalfa leaf.

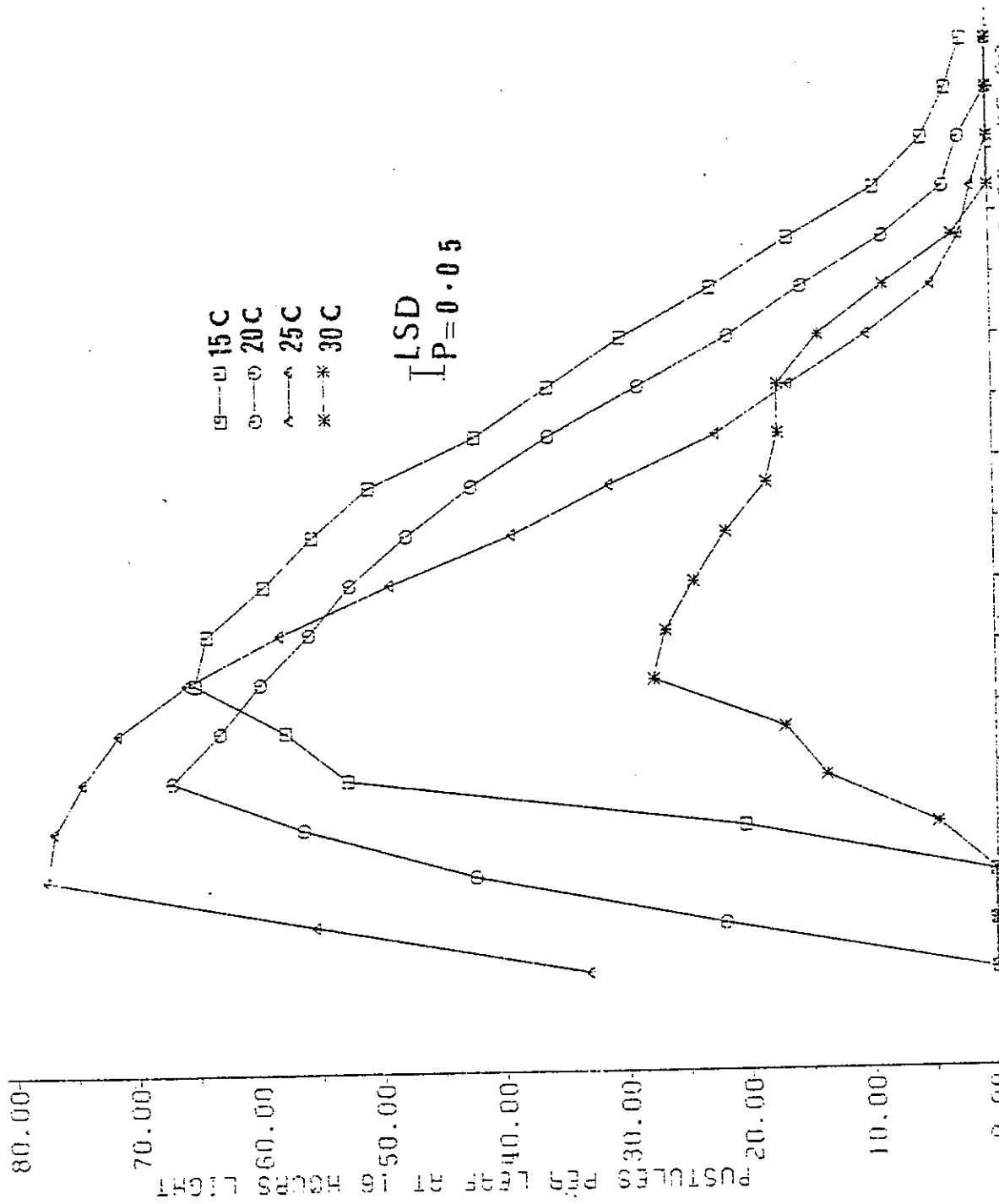

Fig. 6. Effect of four temperatures at a 16-hr photoperiod on the number of sporulating pustules of *Uromyces striatus* per alfalfa leaf.

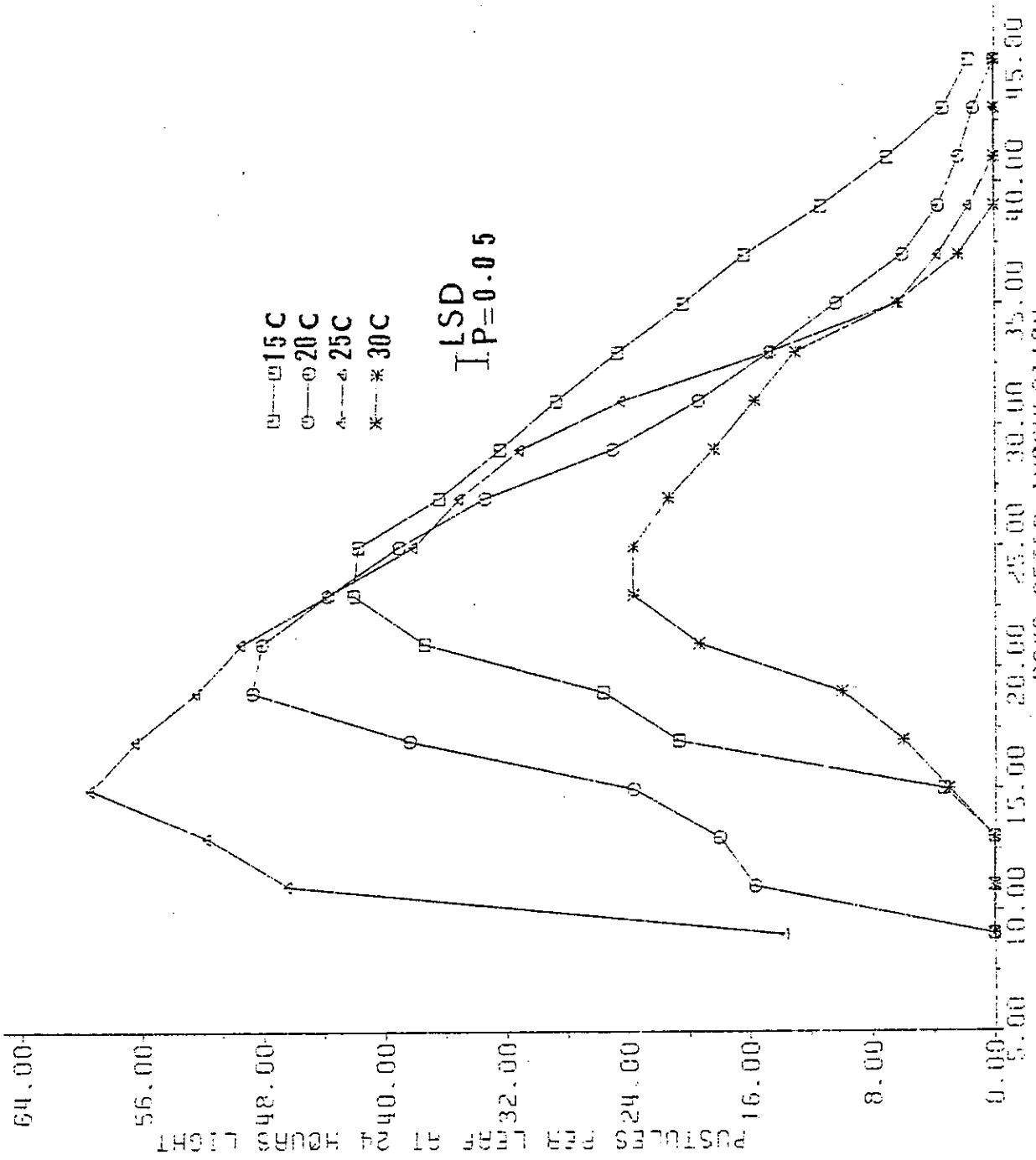

Fig. 7. Effect of four temperatures at a 24-hr photoperiod on the number of sporulating pustules of *Uromyces striatus* per alfalfa leaf.

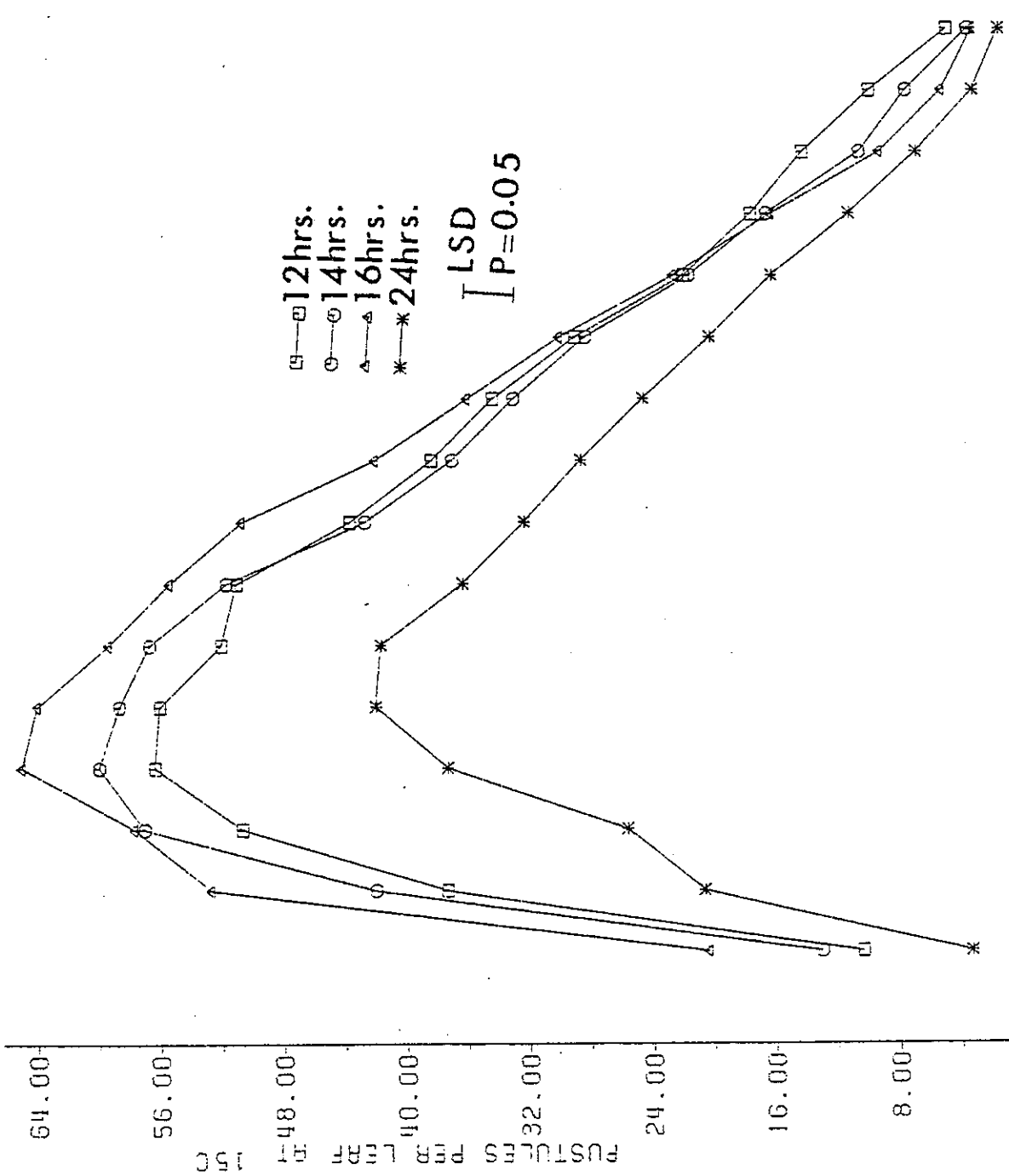

Fig. 8. Effect of four photoperiods at 15 C on the number of sporulating pustules of *Uromyces striatus* per alfalfa leaf.

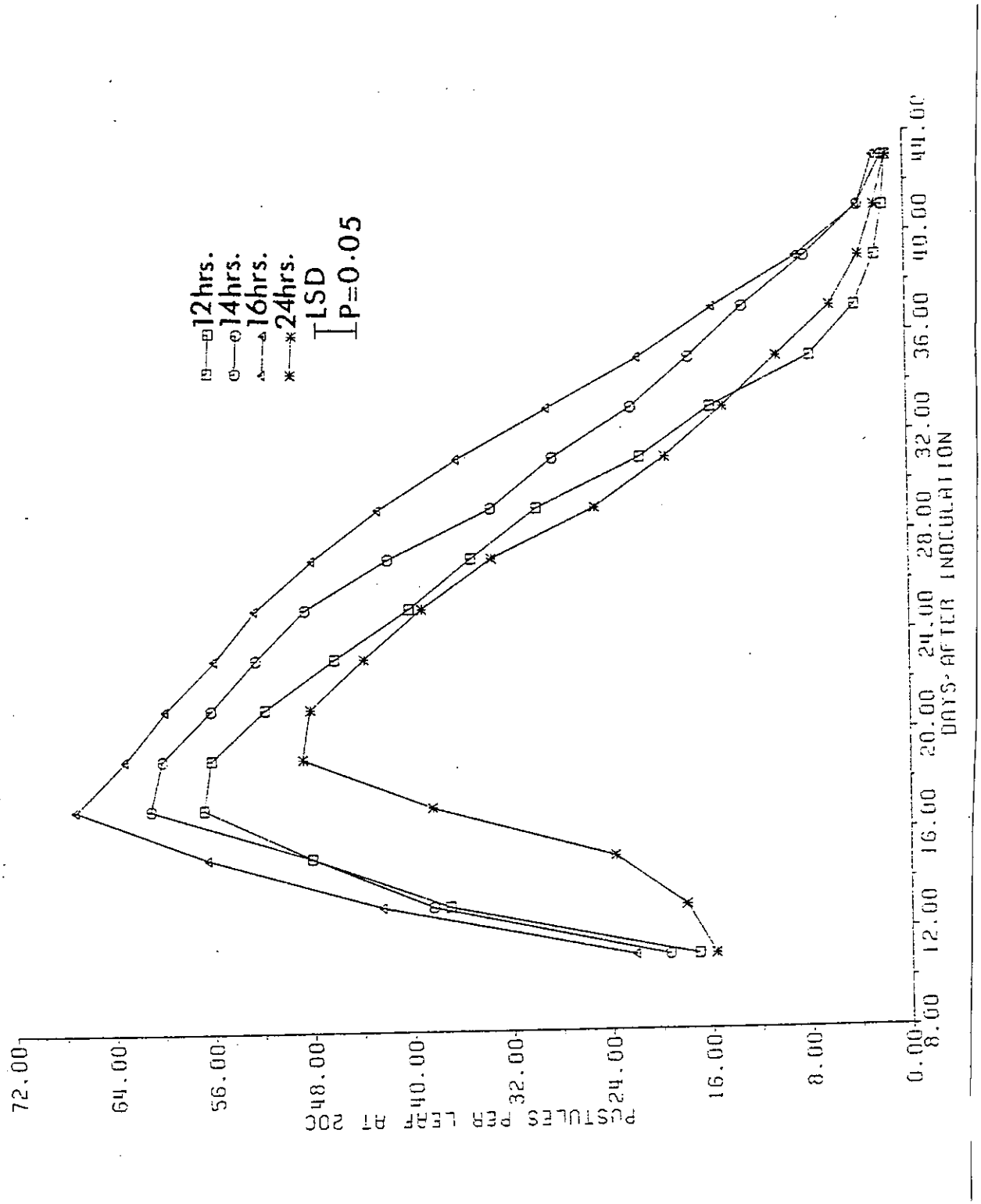

Fig. 9. Effect of four photoperiods at 20 C on the number of sporulating pustules of *Uromyces striatus* per alfalfa leaf.

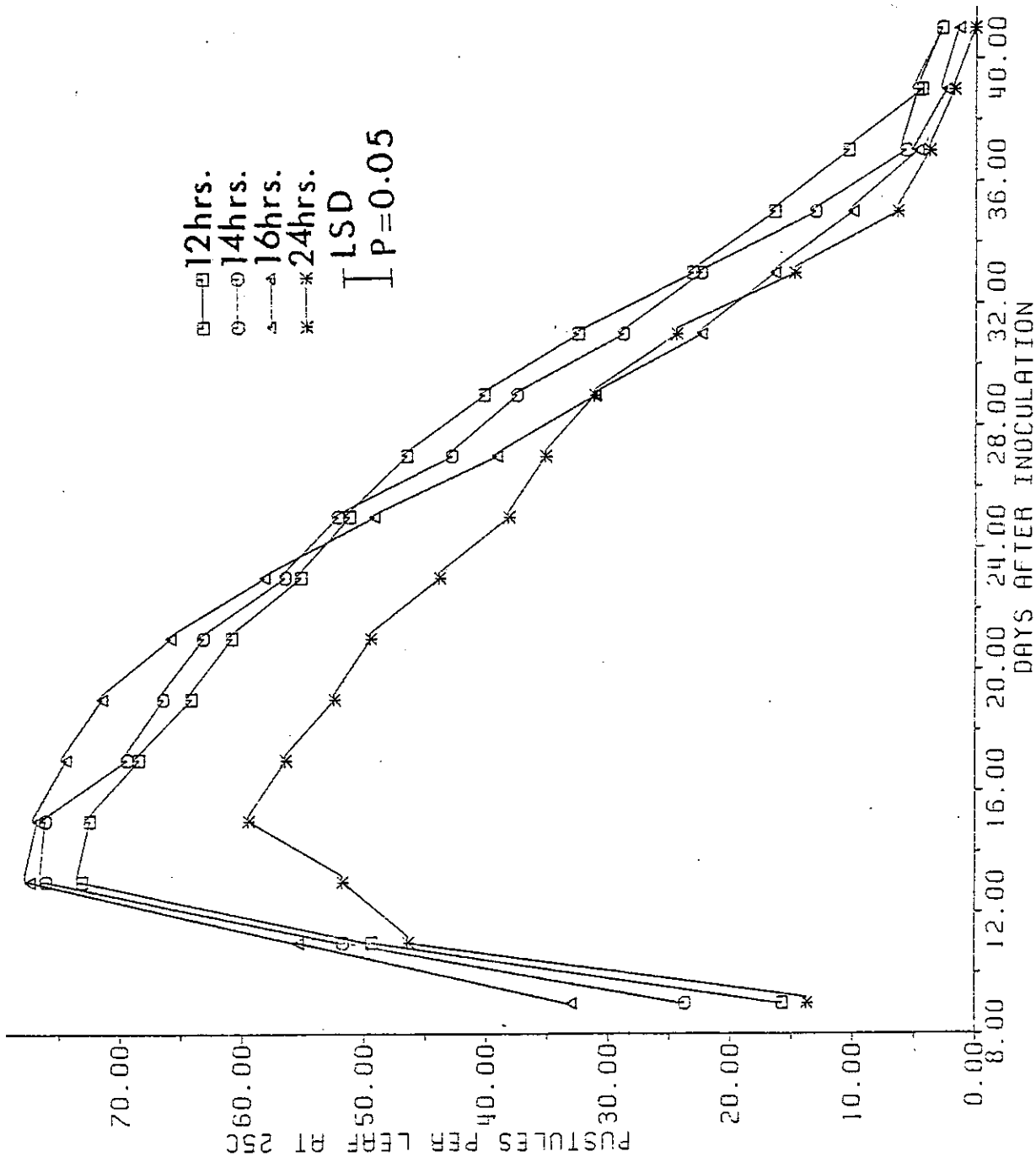

Fig. 10. Effect of four photoperiods at 25 C on the number of sporulating pustules of *Uromyces striatus* per alfalfa leaf.

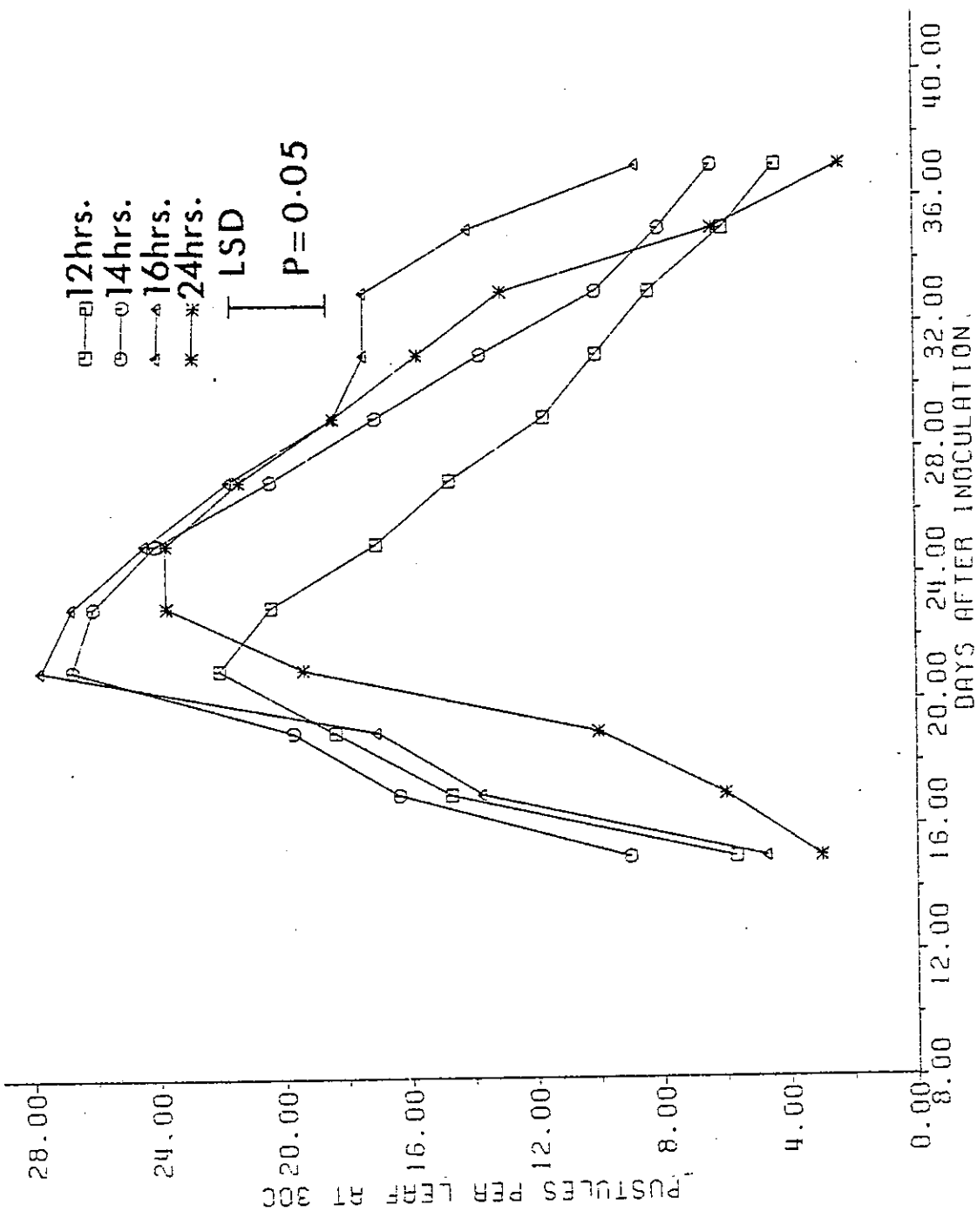

Fig. 11. Effect of four photoperiods at 30 C on the number of sporulating pustules of *Uromyces striatus* per alfalfa leaf.

This shortened the sporulation period to 26 days at the 12, 14, and 16-hr photoperiods and to 24 days under continuous light (Fig. 11). Leaves in all other treatments remained green and turgid throughout the sporulation period.

Spore production. As with pustule numbers per leaf, spore production per leaf was affected more by the temperature range than by the photoperiod range tested (Figs. 12-19) and was significantly greater at 25 C than at any other temperature at all four photoperiods (Figs. 12-15). The total number of spores per leaf at 25 C was 678,696; 638,391; 611,924; and 477,576 [LSD (P = 0.05) = 27,190] at photoperiods of 16, 14, 12, and 24 hr, respectively (Fig. 18). At those photoperiods, total spores produced per leaf at 20, 15, and 30 C were 82-62, 74-56, and 21-16%, respectively, as great as at 25 C. Reduced plant vigor and wilting of infected leaves at 30 C likely contributed to low spore production at that temperature. At all temperatures tested, except 30 C, the highest total number of spores per leaf was produced at photoperiod of 16 hr followed by 14, 12, and 24 hr. At 30 C, the total number at 14-hr photoperiod was greater, although not significantly greater, than at the 16-hr photoperiod.

Teliospores were produced in pustules with urediospores in all treatments. They first appeared 17, 19, 23, and 27 days after inoculation at 30, 25, 20, and 15 C, respectively, regardless of photoperiod and comprised 10-15% of the subsequent spore production. Pustules containing only teliospores were never observed.

Pustule size. After sporulation, four to six infected leaflets from each treatment were removed, placed in test tubes containing 2:1 ethanol-lactophenol, autoclaved for 10-15 minutes and then placed at room temperature overnight. Diameters of 20 isolated pustules (to remove possible effects of pustule density) from each treatment were measured at 100X with a

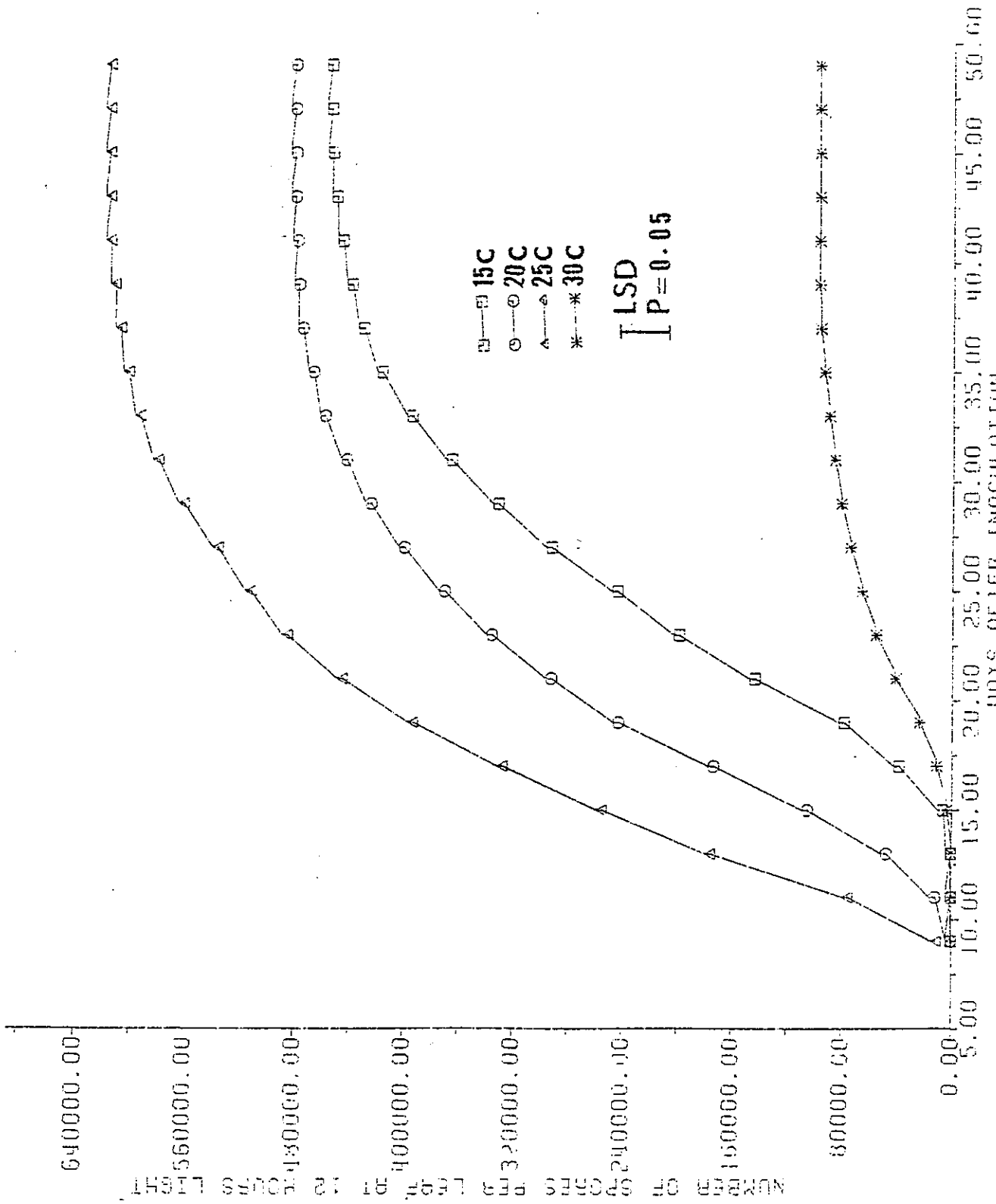

Fig. 12. Cumulative spore production per alfalfa leaf by *Dromyces striatus* at four temperatures with a 12-hr photoperiod.

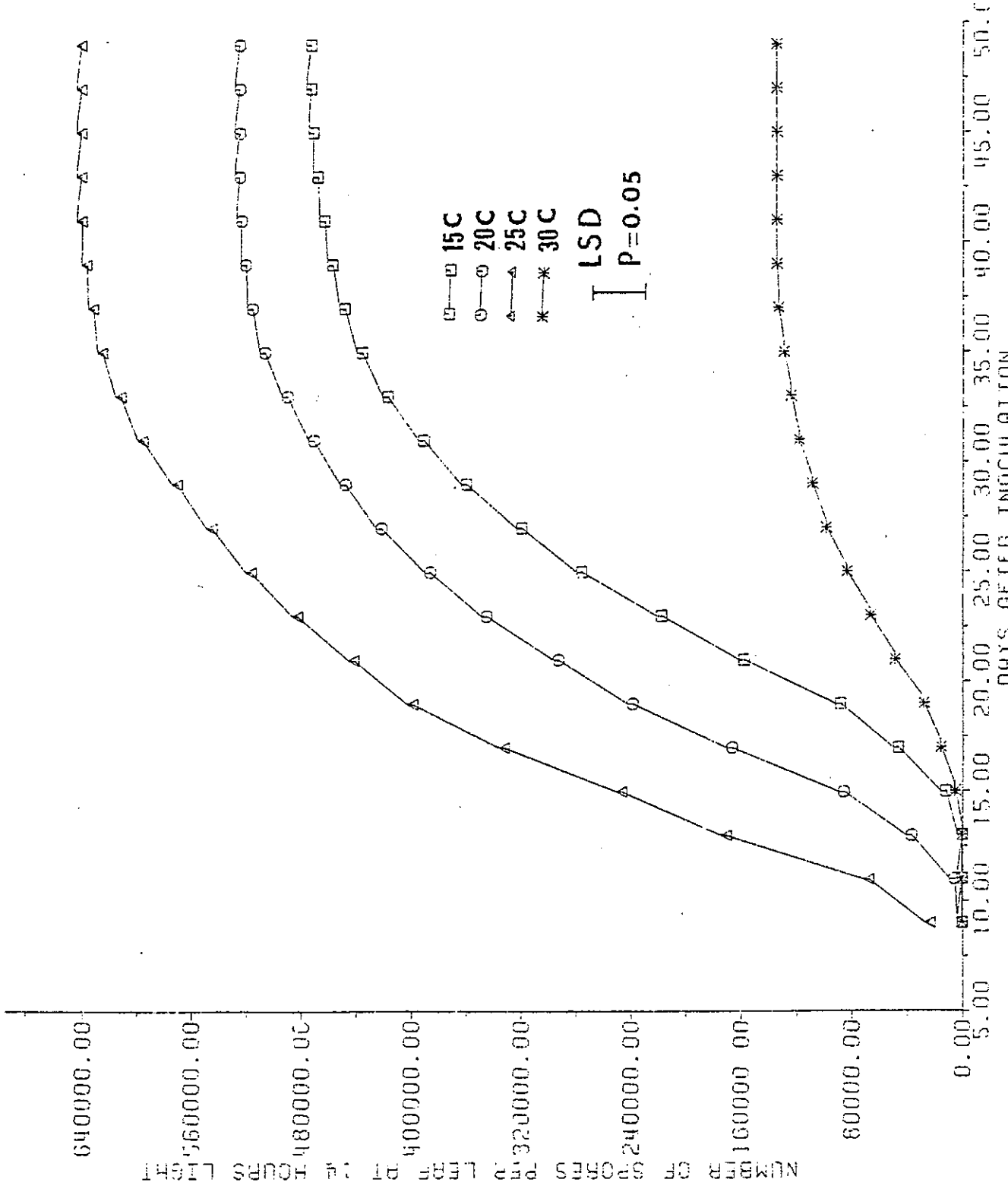

Fig. 13. Cumulative spore production per alfalfa leaf by *Uromyces striatus* at four temperatures with a 14-hr photoperiod.

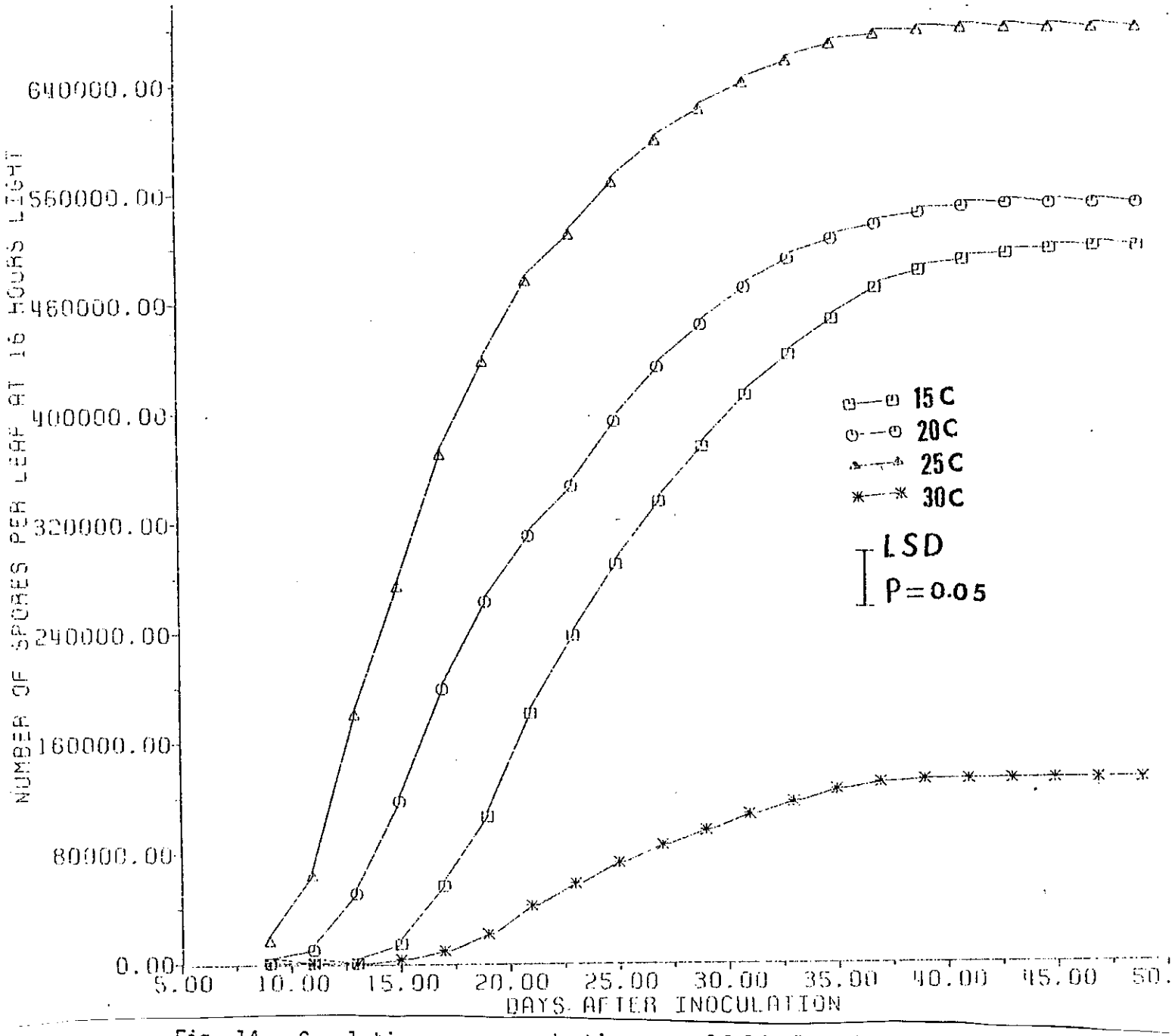

Fig. 14. Cumulative spore production per alfalfa leaf by *Uromyces striatus* at four temperatures with a 16-hr photoperiod.

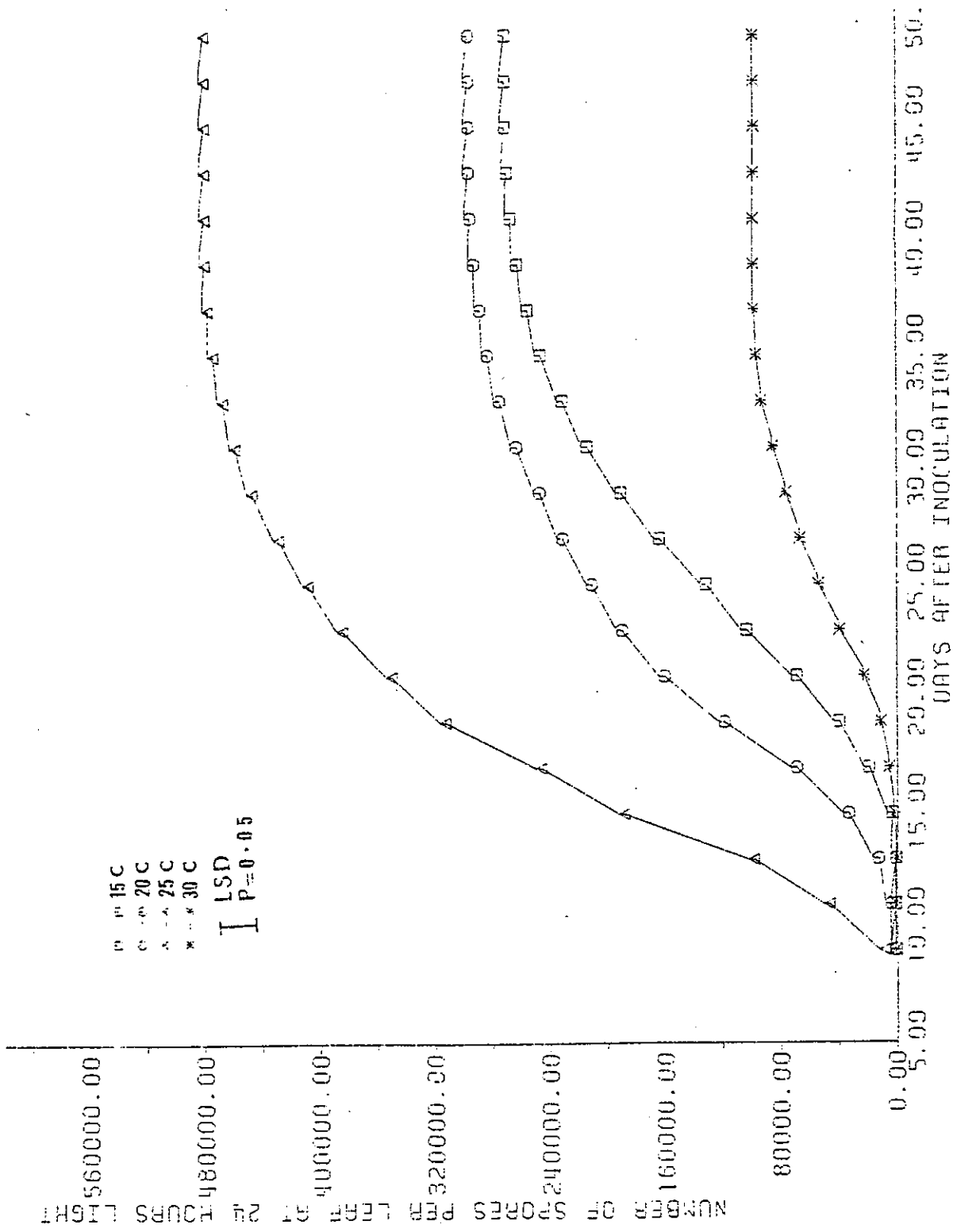

Fig. 15. Cumulative spore production per alfalfa leaf by *Uromyces striatus* at four temperatures with a 24-hr photoperiod.

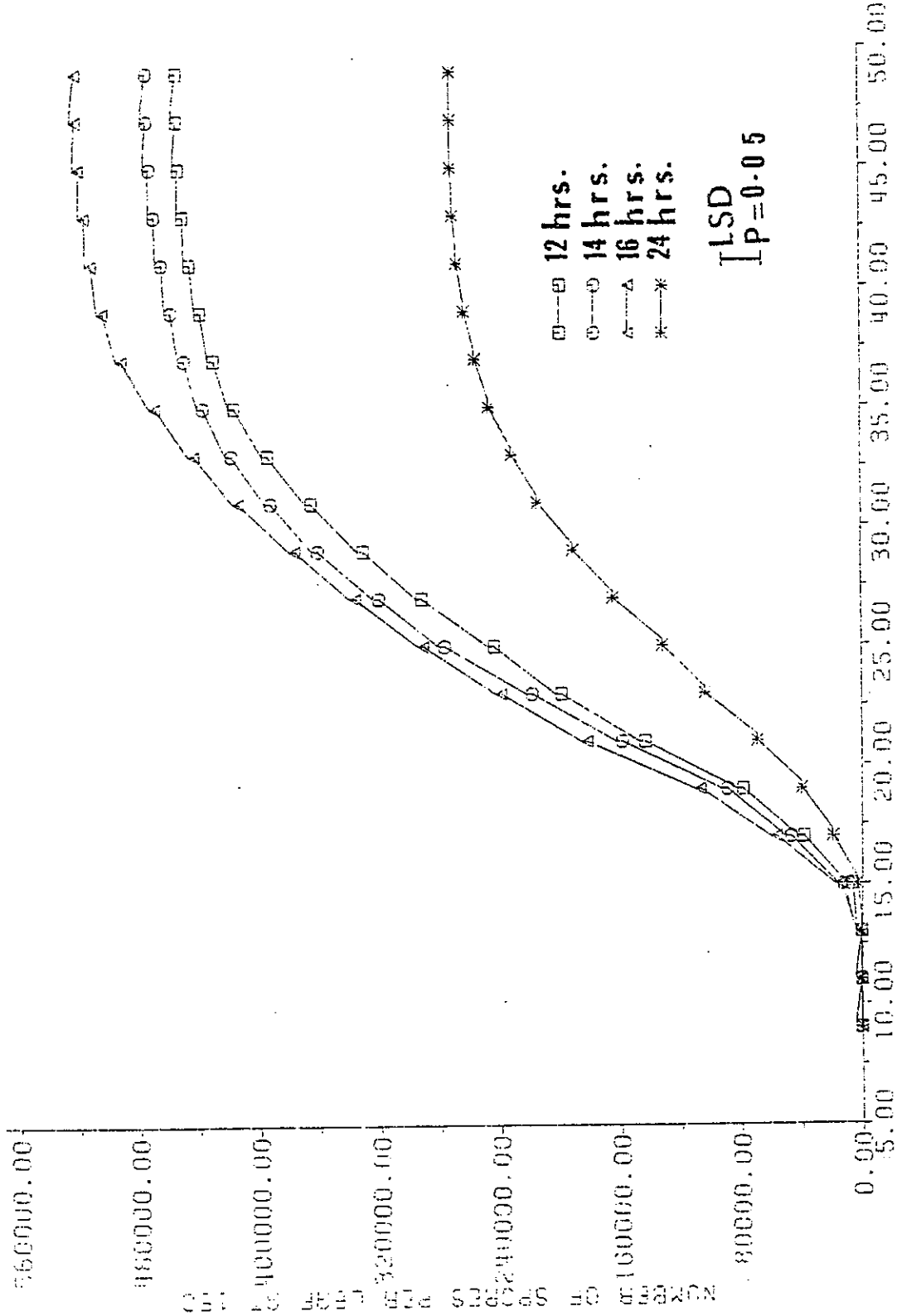

Fig. 16. Cumulative spore production per alfalfa leaf by *Uromyces striatus* at four photoperiods at 15 C.

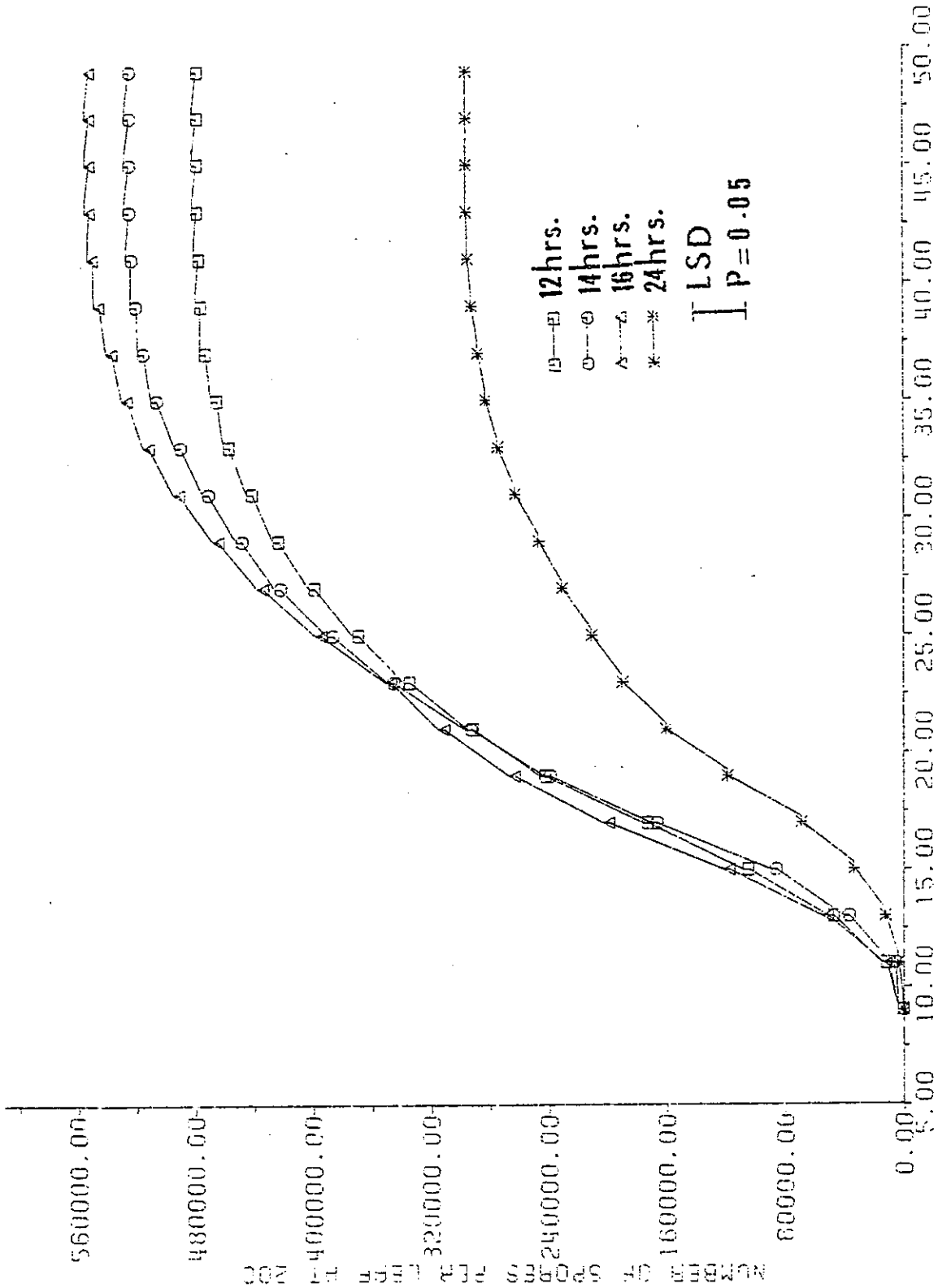

Fig. 17. Cumulative spore production per alfalfa leaf by *Uromyces striatus* at four photoperiods at 20 C.

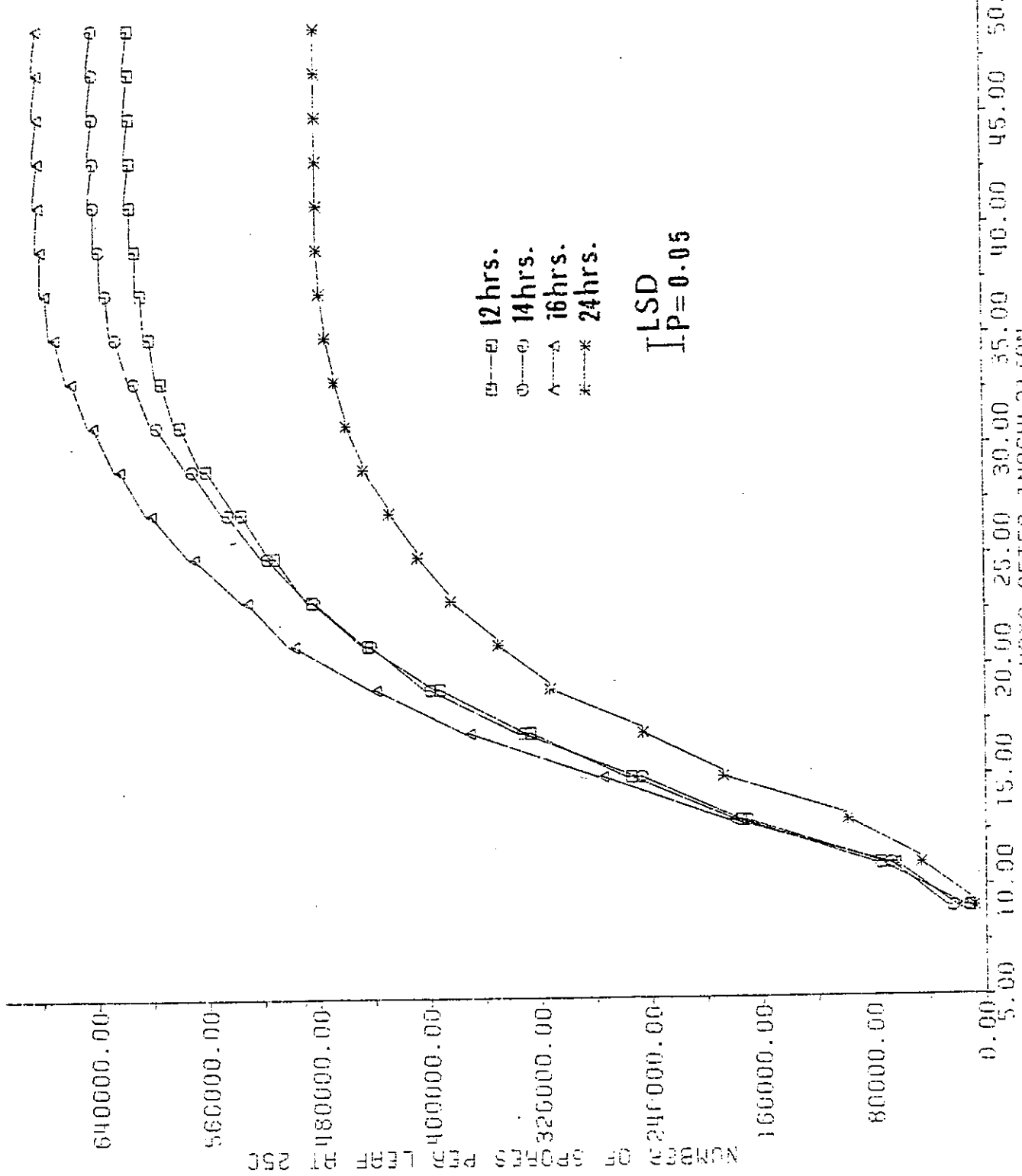

Fig. 18. Cumulative spore production per leaf by *Uromyces striatus* at four photoperiods at 25 C.

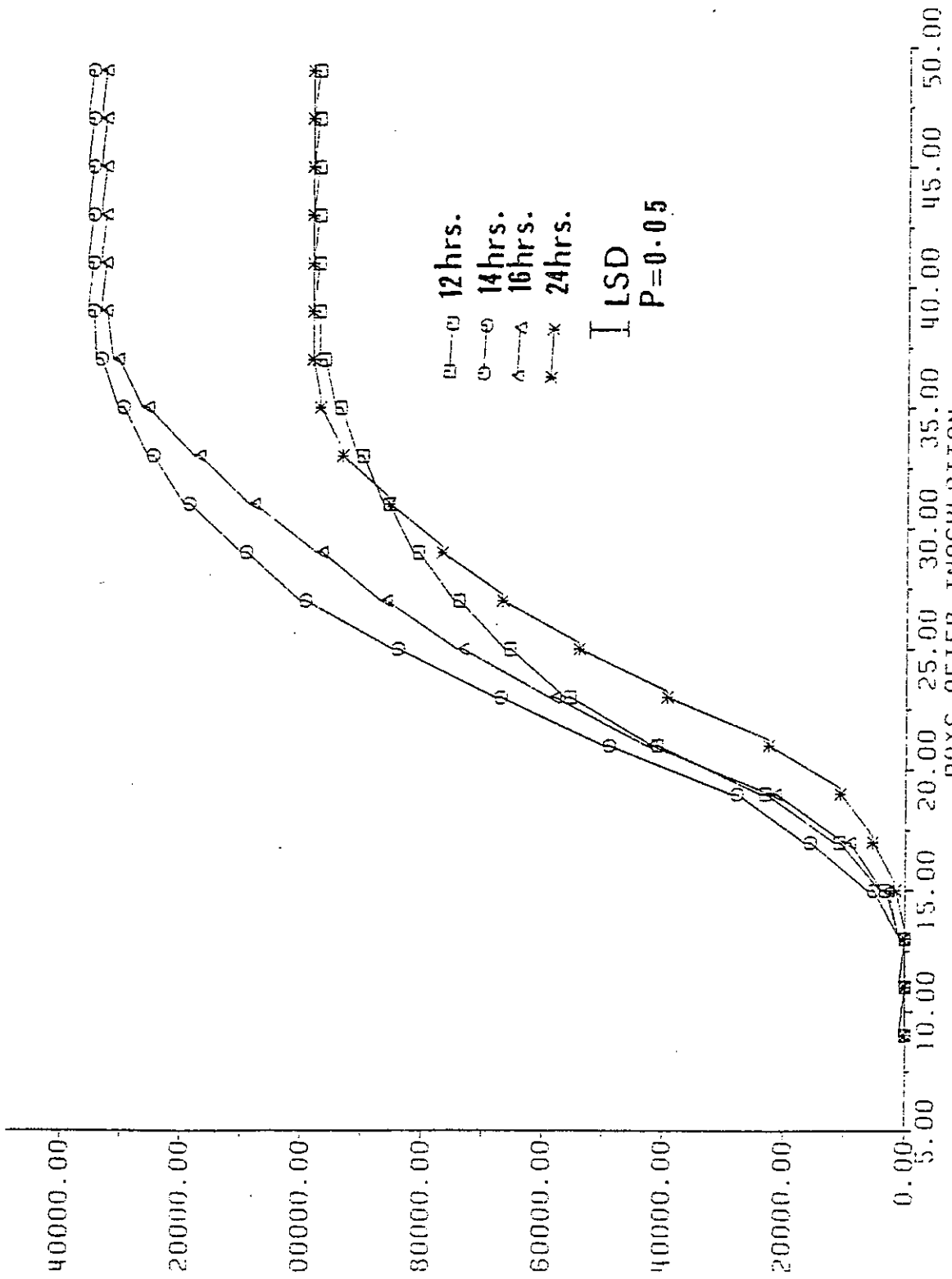

Fig. 19. Cumulative spore production per alfalfa leaf by *Uromyces striatus* at four photoperiods at 30 C.

microscope. Only the actual area of spore production was measured. The mean pustule diameter of 0.49 mm at 20 C and a 16 hr photoperiod was significantly larger than in any other treatment (Table 1). Pustule diameters were the same at 20 and 25 C at photoperiods of 12, 14, and 24 hr. Pustules were smallest at 30 C at every photoperiod.

Effect of postinoculation temperature and duration in mist cabinet on rust pustule production.—To determine the effect of temperature and period of free-water on leaves following inoculation on subsequent pustule production, pots of 20-day-old alfalfa seedlings were placed in mist cabinets at 10, 15, 20, and 25 C immediately following inoculation in a turntable settling tower. At 1-hr intervals for 4-hr and at 2-hr intervals thereafter for 24 hr, one pot (four plants) was removed from each cabinet and placed at 25 C with 8 klx of continuous light. Under these conditions the free moisture on plants evaporated in a few minutes. The experiment was repeated twice.

Pustules developed only on plants kept wet at least 3 hr at 25 or 20 C, 4 hr at 15 C and 6 hr at 10 C (Fig. 20). The most pustules (105 per leaf) occurred on plants kept 16 hr in mist cabinet at 20 C. Next highest number was 92 per leaf on plants kept in mist cabinet 14 hr at 25 C, followed by 15 C for 16 hr (35 pustules per leaf) and 10 C for 14 hr (17 pustules per leaf). Pustule numbers were reduced greatly by keeping plants wet more than 14 hr at 25 C or 16 hr at 20 C.

Effect of leaf age on latent period of *U. striatus*.—Shoots were removed from potted mature plants of a clone of Kanza alfalfa and the plants were placed at 20 C with a 12-hr photoperiod. Every day, for 33 days, emerging leaves were labeled with dated tags. Leaves were approximately 2 days old when their leaflets unfolded. Following inoculation using 20 mg fresh urediospores in the turntable settling tower, the plants were transferred to a darkened mist cabinet at 20 C for 24 hr, and were placed at 25 C with continuous light and observed for pustule development.

TABLE 1. The diameter (mm) of alfalfa rust pustules produced at the indicated temperature and photoperiod combinations

Temp	Photoperiod (hr)			
(C)	12	14	16	24
15	0.42 d*	0.42 d	0.43 cd	0.42 d
20	0.44 c	0.47 b	0.49 a	0.47 b
25	0.44 c	0.47 b	0.47 b	0.47 b
30	0.39 ef	0.41 de	0.40 e	0.38 f

*Means followed by the same letter do not significantly different (P = 0.05) according to Duncan's multiple range test.

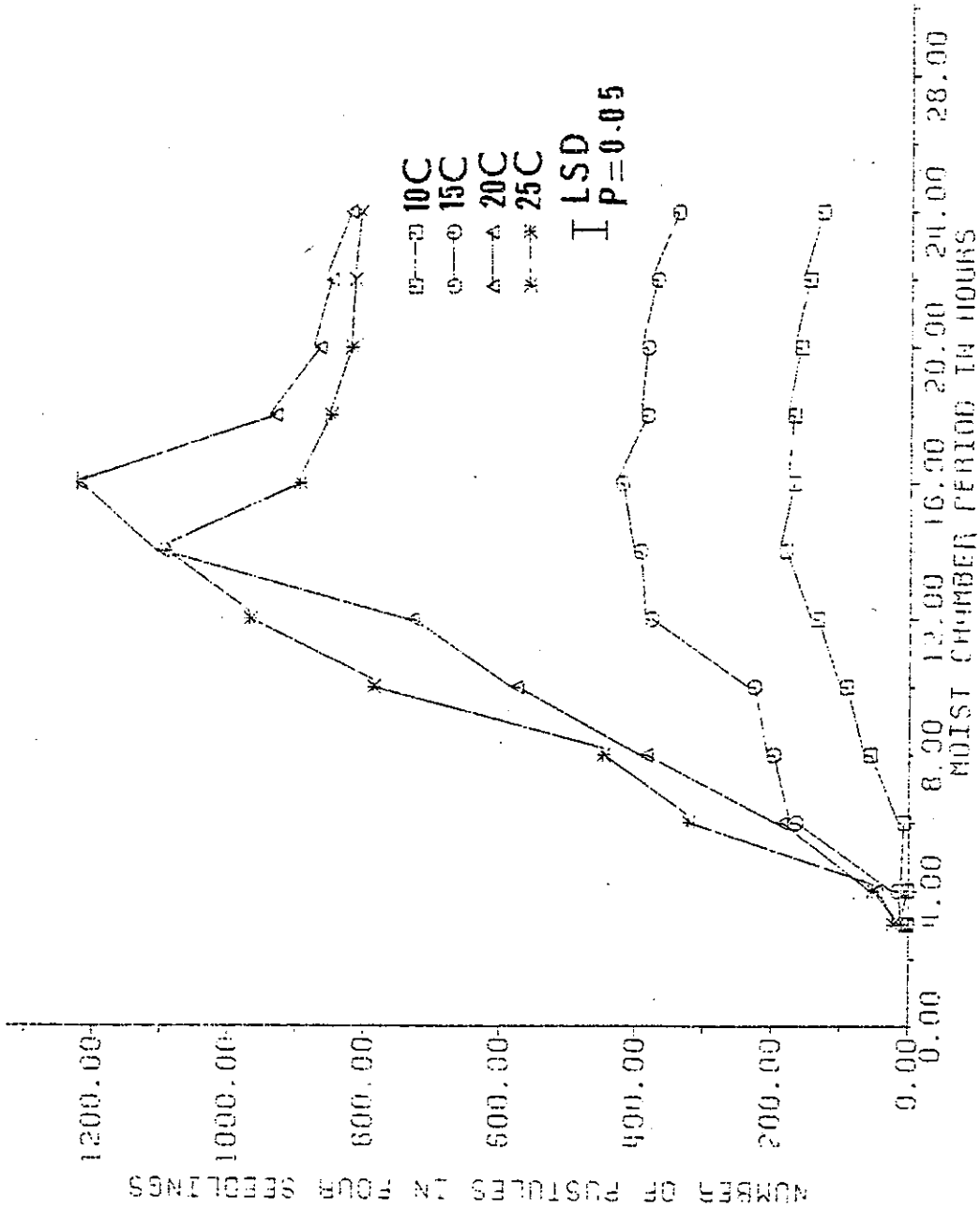

Fig. 20. Effect of post inoculation temperature and duration in mist cabinet on the number of *Uromyces striatus* pustules produced per four 20-day-old alfalfa seedlings (12 leaves).

The latent period was 7 days on leaves inoculated when 5-33 days old and was a half day longer on leaves inoculated when less than 5 days old. Thus leaf age apparently had little if any effect on latent period.

DISCUSSION

Most parameters were affected more by the temperature range than by the photoperiod range tested. Among the 16 postinfection temperature-photoperiod combinations tested at 8 klx light intensity, 25 C with a 16-hr photoperiod most favored alfalfa rust development; the latent period of 7 days was shortest, the sporulation period of 34 days was not exceeded and the 77 pustules and 678,696 spores produced per leaf as well as the 8,814 spores produced per pustule were greatest. However, separate experiments (Fig. 20) indicated that pustule numbers, and likely also spore production, could have been increased with a free-moisture postinoculation period of 16 hr at 20 C rather than the 24 hr used.

Moisture is necessary for infection by *U. striatus*. Most fresh urediospores germinated in water sooner at 15 C than at 25 C (Fig. 1) but time from inoculation to infection, based on the number of resulting pustules, was shorter at 25 C. Temperature requirements were more critical during the postinoculation infection period than afterwards. Urediospores never germinated in water at 30 C and apparently were killed although rust developed on plants placed at 30 C shortly after infection. Plants kept at 15 C during the infection period developed only about 30% as many pustules on those at 25 C (Figs. 4-7). Although a shorter moist period was needed for infection at 25 C than at lower temperatures, more pustules resulted from 16-hr postinoculation moist periods at 20 than at 25 C. The reason for the reduction in pustule production in plants held longer than 14 hr at 25 C or 16 hr at 20 C is not clear. However, drops of urediospore suspensions on slides more than 6 hr commonly included considerable

bacterial activity and lysed germ tubes as reported by Morgan (17) working with some cereal rusts. Perhaps the longer free-water periods enhanced the activity of bacteria and/or other microbes and permitted them to destroy the rust fungus after infection. The fact that the decrease in infection occurred earlier and was greater at the higher temperatures, which most favor bacterial activity, supports that hypothesis.

The validity of using constant temperatures, which obviously do not occur in nature, to interpret or forecast alfalfa rust development in the field is questionable. However, Politowski and Browning (20) produced more *P. coronata* pustules per oat leaf at constant temperatures than with a cam program. Also, changes in one parameter usually affects others. For example, as Mehta and Zadoks (13) pointed out with *P. recondita*, a high pustule density causes a short latent period, a steep increase in daily spore production and a relatively short sporulation period. However, total spore production seems to be relatively independent of pustule density (13, 34).

Environmental conditions favorable for cereal rusts such as caused by *P. coronata* (9, 15) and *P. graminis* (14, 21) also favor alfalfa rust. However, with those favorable environmental conditions, the cereal rusts build-up rapidly in Kansas in the spring, yet alfalfa rust is not reported there until July or August (28, 29, 30, 31). The removal of inoculum with the hay removed at approximately monthly intervals during the growing season would obviously impede rust buildup. That is demonstrated by rust being most severe in stands held for seed production (12). However, the absence of alfalfa rust in the spring is apparently due to the lack of primary inoculum.

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OVERWINTERING OF UROMYCES STRIATUS SCHROET. AND SOME EFFECTS OF TEMPERATURE, PHOTOPERIOD, MOISTURE AND LEAF AGE ON ALFALFA RUST DEVELOPMENT

by

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AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the

requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Plant Pathology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1980

Rust of alfalfa (*Medicago sativa* L.), caused by *Uromyces striatus* Schroet., is usually first reported in Kansas in July or August and, conditions permitting, it builds up until alfalfa shoots are killed by freezing. Origin of primary inoculum in Kansas is unknown and little is known about the environmental requirements for alfalfa rust development. Research was initiated to determine if *U. striatus* urediospores can overwinter in Kansas and to determine some environmental effects, especially temperature and photoperiod, on *U. striatus* development and sporulation.

U. striatus urediospores did not survive beyond January in the field in either 1978 or 1979 but 2.7% of those on shoots harvested October 13, 1978, and stored in an unheated building were viable the following July and some were still pathogenic. However, the latent period (time from inoculation to first erumpent pustule) at 20 C was 14 days in July and 9 days in October.

Urediospore viability decreased from 99% to 13.3% during 23 months storage at -20 C and the latent period at 20 C increased from 9 to 14 days during that period.

Fresh urediospores germinated 42, 39, and 8% by 1 hour at 15, 20, and 25 C, respectively. By 6 hr over 99% germinated at 5 to 25 C but none germinated at 30 C.

To study environmental effects on rust development, potted 20-day-old Kanza alfalfa plants were inoculated with fresh urediospores in a turntable settling tower, placed in a darkened mist chamber for 24 hr at 20 C, and then kept at a light intensity of 8 klx fluorescent lighting at photoperiods of 12, 14, 16 and 24 hr at 15, 20, 25 and 30 C. The mean latent period was 168, 213, 269 and 310 hr at 25, 20, 15 and 30 C, respectively, and was not affected by photoperiod. Sporulation occurred for 34 days at 15, 20 and 25 C at photoperiods of 12, 14 and 16 hr; for 26 days at 30 C

and those photoperiods; and for 24 days at 30 C and a 24-hr photoperiod. Significantly more pustules and urediospores were produced at 25 C than at any other temperature at all photoperiods. Urediospore production was greatest at the 16-hr photoperiod at all temperatures, except 30 C, although the differences at the 16- and 14-hr photoperiods were not always significant. Urediospore production per leaf at 25 C was 678,696; 638,391; 611,924; and 477,567 [LSD (P = 0.05) = 27,190] at photoperiods of 16, 14, 12 and 24 hr, respectively; pustule numbers per leaf were 77, 76, 73 and 59 [LSD (P = 0.05) = 3], respectively. Urediospore numbers per leaf were next greatest at 20 C and were 549,667; 522,423; 476,540; and 294,667 at photoperiods of 16, 14, 12 and 24 hr, respectively. Urediospore production at 15 C was not significantly different than at 20 C. At 30 C, 134,457; 132,228; 97,957; and 96,826 urediospores per leaf were produced at photoperiods of 14, 16, 24 and 12 hr, respectively.

The effects of temperature during infection and length of postinoculation free-moisture period on subsequent pustule production was determined. Following inoculation with fresh urediospores, 20-day-old plants were placed in mist cabinets at 10, 15, 20 and 25 C. At 1-hr intervals for 4 hr and at 2-hr intervals thereafter for 24 hr, pots of plants were removed, placed at 25 C with 8 klx of continuous light, and later observed for rust development. Sporulation occurred only on plants kept wet at least 3 hr at 25 C or 20 C, 4 hr at 15 C, or 6 hr at 10 C. They averaged about 0.8 pustules per leaf. The most pustules (105 per leaf) occurred on plants kept in mist cabinets 16 hr at 20 C. Next highest was 92 per leaf on plants kept wet 14 hr at 25 C. Significantly fewer pustules developed on plants kept wet for longer periods at those temperatures.

Alfalfa leaf age had little affect on rust development. Leaves inoculated when 5-33 days old all developed lesions 7 days later, however, the

latent period was about a half day longer on leaves inoculated when less than 5 days old.