# STUDIES ON THE POWDERY MILDEW DISEASE OF *GALPHIMIA GLAUCA* CAV. CAUSED BY *LEVEILLULA TAURICA* (LÈV.) ARN. AND THE EFFECT OF WATER STRESS IMPOSED ON THE HOST PLANT ON THE FUNGUS.

**A Thesis presented by**

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**LEGON.**

## DECLARATION

I, the undersigned, MARGARET OWUSU, author of this thesis, do hereby declare that the work presented in this thesis,

"STUDIES ON THE POWDERY MILDEW DISEASE OF *GALPHIMIA GLAUCA* CAV. CAUSED BY *LEVEILLULA TAURICA* (LÈV.) ARN. AND THE EFFECT OF WATER STRESS IMPOSED ON THE HOST PLANT ON THE FUNGUS,"

was done entirely by me in the Department of Botany, University of Ghana, Legon, from January, 1997 to September, 1998. This work has never been presented, either in part or completely, for any degree of this University or elsewhere.

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**MARGARET OWUSU B.Sc. (HONS)**

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**EMERITUS PROFESSOR G. C. CLERK**

**(SUPERVISOR)**

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## ABSTRACT

The morphology of the isolate of *Leveillula taurica* parasitizing *Galphimia glauca* at Legon and the effect of water stress imposed on the host plant and on the fungus and infection rate were studied.

*L. taurica* forms dimorphic conidia, conical or primary conidia and cylindrical or secondary conidia. The cylindrical conidia of this isolate were longer than the conical conidia. The two types of conidia were, however, physiologically identical.

The conidia germinated from zero to 100% R.H. Percentage germination in 24 hours of conidia from plants watered daily and with highest Relative Water Content (R.W.C.) was 20.8%. Percentage germination then decreased with decreasing atmospheric relative humidity. Conidia germination was better in light of 76 lux and the germ tubes produced were longer than in dark. Furthermore, conidia formed in light had greater vigour than those formed in dark. Greater germination was stimulated on surfaces of host leaves watered daily. Percentage of conidia obtained from plants watered daily which germinated in 24 hours was 40.0, 38.4 and 22.4 percent when inoculated onto host leaves of 80.6, 74.4 and 66.7 percent R.W.C., respectively.

Water stress imposed on the host plant affected the morphology of the fungus and germination capacity of the conidia. Of the four levels of R.W.C. (80.6, 78.4, 74.4 and 66.7%) maintained in the host leaves, diameter of haustoria, length of unbranched conidiaphores, branching, size of the conidia, level of water content of the conidia, and the germination capacity of the conidia decreased with increasing water stress. Both the R.W.C. of the leaf on which the conidia were formed and the R.W.C. of the leaf inoculated with the conidia influenced infection rate. The best combination was a high R.W.C. of source plant and a high R.W.C. of the inoculated plant.

Infected leaves became chlorotic with 'green islands' and were shed prematurely. The flowers were not infected and no mycelium was detected in the stem.

It was concluded that the *G. glauca* isolate was different in some aspects from the *Capsicum sp.* isolate. Also, because weaker conidia were produced on leaves with greater water stress, infection rate could be reduced by imposing water stress on the plants to a level, which may not unduly harm the plants, but result in the formation of less effective infection propagules.

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# I. INTRODUCTION AND LITERATURE REVIEW.

The powdery mildews are obligate parasites which characteristically grow on the surface of the host plant. The superficial mycelia produce abundant hyaline conidia giving the powdery appearance which gave these fungi the name, 'powdery mildew'. They obtain nutrients from the living host epidermal cells by means of haustoria which arise from the ectophytic mycelium. Depending on the species, powdery mildews may attack any part of the shoot, the stem, leaves, flowers and fruits.

The genus *Leveillula* is unique among the powdery mildews by being entirely endophytic. The intercellular mycelium ramifies among the host cells. During sporulation, conidiophores grow out of the infected organs through the stomata and form the conidia on the host surface (Webster, 1980). The genus *Phyllactinia* is intermediary between *Leveillula* and the rest of the powdery mildews. The vegetative thallus is part endophytic and part ectophytic and the powdery conidia are formed by conidiophores borne by the ectophytic mycelium (Ankora; 1968).

Another outstanding feature of the powdery mildews is the ability of the conidia to germinate over humidities of zero to 100% RH (Yarwood, 1932). The conidia contain high water content which supports germination of the conidia at low relative humidities.

Three genera of powdery mildews, namely, *Leveillula, Phyllactinia* and *Acrosporium (= Oidium)* occur in Ghana. They are economically important as they parasitize crop and medicinal plants (Ankora, 1968; Ayesu-Offei, 1966; Brown, 1976; Debrah-Akomeah, 1990; Tortoe, 1992). From reports worldwide, *Leveillula* is the most important.

*Leveillula* has been recorded on about 1,000 species in 390 genera of 74 families made up of three monocotyledonous and 71 dicotyledonous families. Some of the families have as many as ten or more host species. These are, with the number of host species in parenthesis: Boraginaceae (19), Capparidiceae (11), Caryophyllaceae (16), Chenopodiaceae (41), Cistaceae (22), Cruciferae (20), Euphorbiaceae (41), Labiatae (78), Leguminosae (155), Malvaceae (37), Papaveraceae (78), Polygonaceae (11), Ranunculaceae (15), Rutaceae (17), Scrophulariaceae (38), Solanaceae (37), Urticaceae (45) and Zygophyllaceae (11) (Palti, 1988).

By far, the greatest numbers of hosts of *Leveillula* mildews are herbaceous plants, but there are notable exceptions in various families in which shrubs and trees are also affected. Some of the tree host species are: *Catalpa bignonioides* and *Catalpa speciosa* (Caricaceae); *Quercus* spp. (*Fagaceae); Acacia seyal, Caesalpinia gilliesii, Ceratonia siliqua, Robina pseudoacacia* (Leguminosae); *Morus alba, Morus* sp (Moraceae); *Moringa oleifera* (Moringaceae), *Olea europaea* (Oleaceae); *Passiflora edulis* (Passifloraceae); *Zizyphus jujuba* (Rhamnaceae) and *Prunus amygdalus* (Rosaceae) (Palti, 1988).

Inter-species and inter-genus cross-infectivity is quite common and the reported instances are shown in Table 1.

TABLE 1

**Inter-family cross-infectivity of isolates of *Leveillula* mildews.**

| REFERENCE | FAMILY AND GENUS FROM WHICH INOCULUM ORIGINATED | FAMILY AND GENUS OF CROSS-INFECTED HOST |
|---|---|---|
| Nour (1958) | *Aristolochia* (Aristolochiaceae) | *Euphorbia* (Euphorbiaceae)<br>*Vicia* (Leguminosae) |
| | *Euphorbia* (Euphorbiaceae) | *Hibiscus* (Malvaceae) |
| | *Vicia* (Leguminosae) | *Gossypium* (Malvaceae) |
| | *Gossypium* (Malvaceae) | *Vicia* (Leguminosae) |
| | *Hibiscus* (Malvaceaae) | *Vicia* (Leguminosae) |
| | *Hibiscus* (Malvaceaae) | *Lycopersicum* (Solanaceae) |
| | *Corchorus* (Tiliaceae) | *Vicia* (Leguminosae)<br>*Hibiscus* (Malvaceaae) |
| Besri and Hormattallah, (1985) | *Chenopodium* (Chenopodiaceae) | *Lycopersicum* (Solanaceae) |
| | *Acroptilon* (Compositae) | - ditto - |
| | *Oxalis* (Oxalidaceae) | *Lycopersicum* (Solanaceae) |
| | *Cynara* (Compositae) | *Lycopersicum* (Solanaceae) |
| | *Tropaeolum* (Tropaeolaceae) | - ditto - |
| | *Urtica* (Urticaceae) | - ditto - |

The classification of the *Leveillula* species is uncertain. Two proposals have recently been made to place the taxonomy of *Leveillula* on a firmer basis (Braun, 1980; 1984; Durrieu and Rostam, 1985). Braun (op. cit.) has recognised that for large groups of isolates a clear separation of species is impracticable. He has proposed to unite all these isolates under a collective species, to be referred to as *L. taurica* (Lév) Arn.emend.U.Braun.

However, in addition to this collective species, Braun (Op.cit.) considered a number of the isolates he studied to possess features distinct enough to designate them as separate species, chiefly according to the size of their conidia.

Durrieu and Rostam (1985) have found that the outward appearance of conidia, that is, their ornamentation by networks of ridges and crests, represents a criterion of potential taxonomic significance.

These networks differ in isolates from various hosts in their density and configuration. The above-mentioned concepts were collated to give the following classification:

I. The collective species *L.taurica* (Lév) Arn.emend U.Braun.

II. Species distinguished chiefly by characteristics of their primary conidia.

1. *L. cylindrospora* U. Braun, with conidia cylindrical or ellipsoid, but not pointed; on hosts of the Chenopodiaceae.
2. *L. lactucarum* G. Durrieu and Rostam, with sub-cylindrical conidia bearing a delicate pattern (dense at the tip of the conidium) of ridges and crests; on species of *Lactuca* and *Chondrilla* (Compositae).
3. *L. picridis* (Cast.) G. Durrieu and Rostam, with conidia elongated, cylindrical and tapering at the tip, and with a coarser network of ridges, spaced wider apart than in the other species; on species of *Picris* (Compositae).
4. *L. duriaei* (Lév) U. Braun, with fairly wide conidia bearing a fine, wide meshed network of ridges and spines; on *Phlomis herbaventi* (Labiatae).
5. *L. chrozophorae* U. Braun, with lanceolate conidia and a very dense network of irregular ridges; on *Chrozophora tinctoria* (Euphorbiaceae).
6. *L. rutae* (Jacz.) G. Durrieu and Rostam, with shorter conidia bearing a dense and markedly spiny networks of ridges; on species of *Ruta* (Rutaceae).

III Species distinguished chiefly by their cleistothecial appendages as well as their conidial characteristics.

1. *L. verbasci* (Jacz.) Golovin, with broadly ovoid conidia and numerous very short cleistothecial appendages; on species of *Verbascum* (Scrophulariaceae).
2. *L. saxaouli* (Sorok.) Golvin, with cylindrical conidia with enlarged tops, and with poorly developed, often rudimentary appendages; on Chenopodiaceae.
3. *L. lanuginosa* (Fuck.) Golovin, with dumb-bell shape or sometimes lanceolate conidia, and

numerous well-developed appendages; on Umbelliferae.

All the above taxonomic concepts mark some progress over those proposed earlier, but some practical difficulties still persist. The morphological features used as criteria for distinction between species are unstable and highly host-dependent . Cleistothecia are rare or have never been found on many hosts.

Infection by *Leveillula* mildews produces a marked variety of reactions by the hosts, and hence a wide range of symptoms. The host organ most frequently and often most severely attacked by *Leveillula* is the leaf blade. Petioles, stalks and flower parts are rarely affected and infection of fruits could be regarded as exceptional. Early leaf symptoms are usually apparent on the abaxial side as small whitish areas that gradually expand. On the adaxial side the diseased areas later show yellow colouration which tend to be related to host species. Bright yellow colouration is common in tomato (*Lycopersicum esculentum*), pepper (*Capsicum* sp.), *Cistus* spp, and lucerne (*Medicago sativa*) while creamish areas occur on egg plant (*Solanum melongena*), onion (*Allium cepa*), cotton (*Gossypium* spp.), sesame (*Sesame* sp). and olive (*Olea europaea*). Completely different colouration of reddish-mauve are found on diseased *Gerbera* and *Asclepias* species (Palti, 1988). The white patches appear on both surfaces of the leaves of cotton (Bell, 1981), artichoke (*Cynara scolymus), Cynara cardunculus*, and *Chondrilla juncea* (Rostam, 1983). At the later stages of mildew development, affected tissues turn necrotic in most hosts and the leaves usually drop prematurely.

Infections of petioles have been recorded on hosts of a fair number of families. On parsley, as described by Cirulli (1975), the mildew causes a mealy coverage of the underside of leaves; stems, petioles and flower parts are also affected.

Infection of petioles of parsley has been reported by Cirulli (1975); of flower parts of parsley by Cirulli (1975), safflower (*Carthamus tinctorius*), *Anethum graveolens* and *Chenopodium ambrosioides* by Saad *et* al (1972), and, *Teucrium divaricatum* by Rayss, (1940); and of flowers and pods of fenugreek (*Tricrispum*) by Cirulli (1975).

Boesewinkel (1980) distinguishing between powdery mildews by morphological characteristics of their imperfect state, described *Leveillula taurica* as follows: "Conidiophores appearing in groups of 1-5 through the stomata, 200 - 700 x 3.7 - 7.4 μm, with 2-3 branches which are up to 110 - 260 μm long. Conidia cylindric, clavate or avoid, 40 - 80 x 12 - 16 μm, producing a moderately long germ tube at the end, rarely at the side. Appressoria multilobed or branched, double or single. Haustoria globose, moderately lobed, 15 - 30 μm wide. Mycelium dense and conspicuous on a large number of plant families". Observations made and recorded in this thesis agree with those of Boesewinkel (op. cit) and, therefore, identify the fungus of this investigation as *Leveillula taurica*.

*Leveillula taurica* was first recorded in Ghana in 1958 (Leather, 1959). Since then, a lot of work has been done on isolates from pepper (*Capsicum* spp.) (Ayesu-Offei, 1966; Brown, 1976; Caesar, 1979). Isolates of three other hosts which have been studied locally are egg plant (*Solanum melongena*) (Hughes, 1953; Leather, 1959; Pienning, 1962,), tomato (*Lycopersicum esculentum*) (Clerk, 1974) and the spinach (*Solanum macrocarpon*) (Brown and Clerk, Unpublished Report-personal communication).

The environment of a biotrophic pathogen is principally biotic, determined mainly by the living host plant. Abiotic factors such as water supply, are mediated by the host (Wyness and Ayres, 1985).

Brown (1976) observed that pepper host varieties had different effects on the morphology of *L. taurica*. Differences in effect of host variety were observed in haustorial size, percentage of conidiophores that were branched, length of the unbranched conidiophores and the maximum number of conidia produced by a generative cell. For instance, whereas the size of conidia of *L. taurica* produced in the cells of his large-fruited pepper variety ranged from 13.3 - 19.9 μm and 9.9 - 16.7 μm in length and width, respectively, that of the Round-fruited variety ranged from 9.9 - 16.6 μm and 9.9 - 13.3 μm in length and width, respectively, with the sizes of the conidia from other pepper varieties falling between these two. Brown (op. cit.) also found that branching conidiophores of isolates infecting the Large-fruited, Long-fruited, Medium-fruited, Round-fruited and Small-fruited varieties formed only 6.8, 11.5, 10.1, 1.9 and 7.4 percent, respectively, of the total number of conidiophores observed. Furthermore, the isolate of the small-fruited variety showed the longest mean conidiophore length of 230.3 μm compared to the mean conidiophore length of 176.2 μm of the isolate on the Large-fruited variety.

Brown (1976) also reported that *L. taurica* conidia from pepper plants watered with half strength, standard and double-strength Sach's Solution were similar in size, but showed different germination capacities. Those from plants raised on Standard Sach's Solution germinated better and produced longer germ tubes at 100% R.H. than conidia from plants of the other two treatments. Percentage germination of 66.3, 64.1 and 56.9, respectively, was obtained for *L. taurica* conidia produced by mycelium growing on Long-fruited pepper plants supplied with standard, half-strength and double strength Sach's Solutions.

Caesar (1979) studied the effect of water potential on growth of *Capsicum annuum* plants and development of *L. taurica*. He found out that water stress ranging from -11 to -24 bars imposed on *C. annuum* plants from seedling stage affected both growth of the plants and morphology and physiology of *L. taurica* parasitizing the plants. Plants of greater moisture content and apparently with better nutrient content were more susceptible to attack by *L. taurica*. First, it took 9 - 10 days for the lesions to appear on plants kept at moisture level of -5.5 bars and 11 - 13 days on plants of water stress of -24 bars (Caesar, 1979).

Secondly, the conidiophore length, conidial size and conidial germination capacity decreased with increasing water stress. Thus, the mean conidiophore length, mean length of the cylindrical conidia and the mean maximum germ tube length formed on glass slides at 100% R.H. by germinating conidia of mycelium on plants at -5.5 bars water potential were 248.8, 69.7 and 215.5μm, respectively. The corresponding values by mycelium on plants of -24 bars water potential were 158, 53.3 and 119.6μm respectively.

The effects of the host on other powdery mildews have also been studied. Studies showed that older plants of barley (*Hordeum vulgare*) plants were less affected by *Erysiphe graminis* DC f. sp *hordei*. The plants showed fewer number of colonies of the mildew which grew slowly and sporulated rather poorly (Woolacott and Ayres, 1984).

Ayres (1977) investigated the effect of water potential of pea leaves on sporulation by *Erysiphe pisi* DC. He reported that water stress imposed on field peas by the cessation of watering at different times before or after inoculation caused decreased mycelial growth and conidial production as leaf water potential decreased from -4.5 bars (soil water at field capacity) to the permanent wilting point (-9.5 bar). He obtained mycelial growth of 1.1mg measured as fresh weight per cubic centimetre of leaf surface area on leaves of field pea at -4.5 bars water potential, but this decreased to 0.9 mg at -9.5 bars. Spore production also decreased from $30 \times 10^3 cm^{-2}$ to $12 \times 10^3 cm^{-2}$.

Woolacott (1982) reported that growth of wheat host plants under water stress had no effect upon efficiency of haustoria of *Erysiphe graminis* f. sp. *hordei* but affected the size of haustoria on leaves of plants of different ages. This according to Woolacott and Ayres (1984), contributed to some extent to the growth and productivity of the fungus.

The germination of conidia of the powdery mildews could be affected by other host factors. While Brodie (1945), Cherewick (1944) and Yarwood (1932) secured significant or high germination of *E. graminis* conidia from barley at 0% R.H., Clayton (1942) and Grainger (1947) obtained no germination at 0% R.H. with conidia of *E.graminis* from *Hordeum*, *Triticum, Avena* and *Poa*. According to Yarwood (1957), high temperatures reduce the tolerance of powdery mildews to low humidity and this might explain the failure of some investigators to obtain germination of *E. graminis* conidia at low humidities.

Host effect and the influence of environmental conditions on sporulation and germination capacity of spores have been observed in many other groups of fungi. For example, Leonian (1927) provided one of the most illustrative examples of host effect on sporangial morphology. He inoculated fruits of banana (*Musa sapientum* Linn.), egg plant (*Solanum melongena* Linn.), lemon (*Citrus medica* L., var *limonum*), orange (*Citrus aurantium* Linn.) with *Phytophthora capsici* Leonian, *Phytophthora phaseoli* Thaxter, *Phytophthora omnivora* de Bary and *Phytophthora mexicana* Hotson and Hartge. All the hosts were infected almost to the same degree but sporangia were produced in great abundance only on tomatoes, egg plants and peppers, and the sporangia showed striking differences in shape and nature of their papillae according to the host species. The fungi commonly formed small-sized sporangia on egg-plant. Shapes of the sporangia included numerous forms, typical lemon shape, globular, pyriform, ellipsoidal and filiform shapes. On tomato, the sporangia of *P. mexicana*, for instance, were small and globular while those on egg-plant and pepper were ellipsoidal and large. The germination capacity and physiology of the various forms were not investigated.

An interesting further observation is the relationship between the position of a spore on the sporulating sporangium and its size and germination capacity. Asomaning (1977) studied sporangium production in *Phytophthora palmivora* (Butl.) She observed that the size of the sporangia decreased with successive sporangia on the sporulating branch. For example, on branches each with six sporangia, the mean sporangial lengths from the oldest (at the base) to the youngest (at the apex) were 45.0, 40.6, 35.4, 28.6, 27.6 and 21.3μm, respectively. Furthermore, sporangia less than 24.5μm long (youngest), between 24.5 and 48.5μm long (medium) and more than 48.5μm long (oldest) showed indirect percentage germination (by means of zoospores) of 72.1, 89.7 and 6.0 percent, respectively, in two hours.

What was observed by Asomaning (1977) had earlier been noticed by Clerk (1963). Interestingly, the three fungi (*Beauveria bassiana, Isaria farinosa* and *Metarrhizium anisopliae*) studied by Clerk (op.cit.) differed in spore position-spore germination capacity relationship. In *M. anisopliae*, germination capacity of the conidium was not related to its position in the chain. All the conidia in a chain germinated to the same degree. *B. bassiana* and *I. farinosa*, which clearly showed conidium position-germination capacity relationship showed contrasting, patterns. Whereas in *B. bassiana* germination was better in younger conidia on the phialides, older conidia germinated better than younger ones in *I. farinosa*. In both cases, all conidia on a sporulating branch were formed in less than 24 hours.

A new powdery mildew disease by *Leveillula taurica* has recently been discovered in Ghana (Clerk, Personal Communication), and the host plant is also a new one for the fungus and was not listed by Palti (1988) nor reported elsewhere after Palti's (op.cit.) publication. The isolate occurs on *Galphimia glauca* Cav. (Malpighiaceae), an ornamental plant widely grown in gardens and used also as a hedge.

Large gardens are not normally watered in Ghana and *G. glauca* plants are subjected to the usual wet and dry conditions corresponding to the rainy and harmattan seasons. Water available to the plants, therefore, fluctuates and the fungus, growing on the plants would also be subjected to some amount of water stress in the dry season.

This project was carried out to study among other things, the effect of different levels of moisture content of the plant on the fungus. In the study carried out by Caesar (1979) on *L. taurica* infecting pepper host plants, mentioned earlier in this review, the plants were subjected to different levels of water stress right from the seedling stage. The plants under severe water stress were diminutive and the fungus was also shown to be diminutive. Under natural conditions, crops do not normally grow under unbroken water stress. The results of the study, therefore, did not show how the fungus on plants growing under normal moisture conditions would be affected by brief water stress conditions as would naturally occur. This investigation was designed to study the behaviour of *L. taurica* under such conditions.

Palti (1988) pointed out that *Leveillula* mildews appear to be dynamic in nature, spreading constantly to new regions and new hosts. He stated that they also seem to increase in pathogenic intensity on certain crops. Whether or not the discovery of this new host (*G. glauca*) of *L. taurica* is due to introduction of a new host variety and cultural practices, or to the appearance of new *L. taurica* races or to all these factors, remains to be elucidated. Studies on this new disease and the *L. taurica* isolate would, therefore, show how similar it is to, or how different it is from, the other isolates which had been studied in Ghana.

The work was carried out to study principally:

1. the epidemiology of the powdery mildew disease of *Galphimia glauca.*
2. the effect of light on germination of the conidia.
3 the effect of water stress imposed on already infected *G. glauca* plants on growth and morphology of the mycelium developing after the application of the treatment.
4. the nutrient and water content of *L. taurica* conidia formed after imposition of different moisture conditions.
5. the germination habits of conidia formed by *L. taurica* subjected to different moisture conditions.

## II. MATERIALS AND GENERAL METHODS



### (i) MATERIALS

a. Host plant

*Galphimia glauca* plants used in this investigation were raised from young seedlings obtained from a plot in front of the Balme Library of the University of Ghana. Seeds collected from the plants on several occasions germinated very poorly in the laboratory, probably due to dormancy, and could not provide adequate numbers of seedlings required.

b. Strain of *Leveillula taurica*.

The strain of *Leveillula taurica* used was obtained from naturally infected leaves of *Galphimia glauca* plants growing on the plot in front of the Balme Library of the University of Ghana.

### (ii) GENERAL METHODS

a. Raising of Experimental Plants.

Only seedlings at the 4 - leaf stage and showing no signs of infection were selected. They were transplanted into black plastic bags measuring 20cm wide and 27cm long, containing garden loam soil, and with drainage holes at the bottom.

The plants were watered daily for three days before commencement of any treatment to allow them to overcome the shock of transplanting and to allow the roots to become established in soil. The plants were put on the eastern verandah of the research laboratory and therefore received direct sunlight for five hours each day, and were in the shade for the rest of the day.

b. Establishment of different relative water content of the leaves.



The growing plants were divided into four batches and given different amounts of water to establish different relative water content of the leaves:

Batch 1: plants in each pot were watered daily with 50ml of distilled water.

Batch 2: plants in each pot were watered each other day with 50ml of distilled water.

Batch 3: plants in each pot were watered once every three days with 50ml of distilled water.

Batch 4: plants in each pot were watered once every four days with 50ml of distilled water.

c. Determination of Relative Water Content (RWC) of leaves.

The method for the assessment of the relative water content (RWC) of leaves devised by Weatherly (1950, 1951) was used to determine the relative water content of the leaves of plants of the four batches, which received different quantities of water. The parameters determined and used were leaf fresh weight, leaf turgid weight and leaf dry weight.

The RWC was calculated using the formula of Weatherly (1950, 1951):

$$RWC = 100 \times \frac{\text{Fresh weight - Dry weight}}{\text{Turgid weight - Dry weight}}$$

d. Determination of Leaf Fresh Weight.

Leaf-discs were removed with a 6mm-diameter cork borer from plants of each treatment and weighed immediately, to provide the leaf fresh weight.

e. Determination of Leaf Turgid Weight.

Leaf discs were removed with a 6mm-diameter cork borer from plants of each treatment and floated in de-ionized water in Petri-dishes for six hours. They were then removed quickly, surface-blotted with tissue paper and weighed immediately.

f. Determination of Leaf Dry Weight.

Leaf discs were removed with a 6mm-diameter cork borer from plants of each treatment and dried in an oven at 80°C for three days. At the end of the second day, the Petri dishes containing the leaf discs were removed, placed in a desiccator to avoid absorption of moisture and the leaf discs quickly weighed and returned to the oven. They were re-weighed twice on the third day at 12.00 Hours GMT and at 15.00 Hours GMT to ensure that a constant dry weight had been achieved.

Five batches of 10 leaf-discs each were used for each weight determination.

Leaf-discs as recommended by Weatherly (1950, 1951) were used as leaf-disc saturation has advantages over the saturation of whole leaves (Weatherly 1950, 1951; Slavik, 1974). Whole leaves take several hours, 24 to 48 hours, to attain full saturation (Slavik, 1974). According to Slavik the experimental material respires during this interval resulting in a loss of dry weight. Leaf-discs floated on water become rapidly saturated within 4 hours (Catsky, 1965)

g. Inoculation of test plants.

Test plants for parasite-host water content relationships were inoculated by gently adpressing actively sporulating lesions against the abaxial surfaces of the leaves. In each case the leaf was inoculated at the centre, so that disease progress could be followed in any direction for reasonable length of time.

h. Humidity Chambers.

(i) Van Tieghem Cells.

Solid watch glasses, measuring 3.7 x 3.7 x 1.6cm with a well 3.5cm in diameter and 1.0cm deep, were used as Van Tieghem Cells for most of the conidial germination tests requiring specific constant relative humidities. An amount of 2ml of the appropriate solution (5.0g in the case of solid reagents) was put into the well of each watch glass to maintain the desired relative humidity. The top edge of the watch glass was smeared with petroleum jelly to form an air-tight seal when the lid was placed on it. *Leveillula taurica* conidial print was made at the centre of the cover glass within a circumscribed area of 1.0cm in diameter of the glass lid by gently adpressing the mildewed area to the glass lid. The lid was then placed on the watch glass with the conidium - bearing side facing downwards.

(ii) Petri dishes.

Where a humidity of 100% R.H. was required, spore prints on glass slides were incubated in humid atmosphere in Petri dishes. About 2ml of distilled water was put in a clean Petri dish to provide 100% R.H. The slide with the spore-bearing side facing upwards was then placed on a V-shaped glass rod in the Petri dish and the dish then covered with the lid.

i. Maintenance of Constant Humidities.

Constant relative humidities ranging from zero to 92.8% R.H were maintained in the Van Tieghem cells with solutions of glycerol of different concentrations prepared according to the data of Solomon (1952), and as shown in Table 2.

Table 2: Glycerol Solutions for maintaining constant humidities. (Data of Solomon, 1952).

| % Relative humidity | Weight of Glycerol (g) | Volume of Water (ml) |
|---|---|---|
| 0.0 | 50.0 | 0.0 |
| 16.9 | 46.0 | 4.0 |
| 44.8 | 37.5 | 12.5 |
| 62.5 | 30.0 | 20.0 |
| 85.2 | 17.5 | 32.5 |
| 92.8 | 12.5 | 39.5 |

j. Preparation of conidial prints for germination test.

Glass slides and glass covers of solid watch glasses were gently brought into contact with the lesions on the abaxial surface of the diseased leaf and quickly transferred to the Petri dishes and solid watch glasses, respectively.

k. Assessment of condium germination.

Conidia incubated on glass slides or solid watch-glass lids were examined immediately at the end of the desired incubation period without staining. A conidium with discernible germ tube was considered to have germinated.

Assessment of degree of conidium germination for each treatment was based on the percentage of three hundred observed conidia. The length of the germ tubes of the germinated spores was also measured and the mean germ tube length for the treatment calculated. The mean germ tube length was based on 20 measured germ tubes. Measurements were made with an eye-piece graticule. Where a germ tube branched, germ tube length was taken as the sum of the length of the main germ tube and those of the branches.

l. Comparison of moisture content of conidia.

The moisture content of conidia was compared using the shrinkage method, that is, the rate at which water was lost from the conidia.

About 5g of anhydrous $CaCl_2$ was put into the well of solid watch glasses measuring 3.7 x 3.7 x 1.6cm. The top edge of the watch glass was smeared with petroleum jelly to form an air-tight seal when the lid was placed on it. *Leveillula taurica* conidial print was made at the centre of the cover glass by gently adpressing the mildewed area to the glass lid. The lid was then placed on the watch glass containing the anhydrous $CaCl_2$ with the conidium bearing side facing downwards. At the same time a stop watch was started. The total number of conidia in a high power field of the microscope, as well as the number of shrunken and unshrunken conidia were recorded every ten minutes until the percentage of shrunken conidia remained constant. This was done in duplicate for conidia obtained from plants with leaves of different Relative Water Content.

m. Germination of conidia in light and dark.

Fluorescent tubes (40 - watts) in the laboratory provided the light source. Petri dishes containing glass slides with conidial prints as described in section (g) were arranged on the laboratory bench. The intensity of light from the fluorescent tubes falling normally on the bench on which the set-up was arranged was measured with a light meter.

Where different intensities of light were required, the photosensitive cell of the light meter was covered with different thicknesses of tracing paper on the laboratory bench and the intensity of light falling on the photosensitive cell recorded for the one sheet, two sheets, three sheets and four sheets thickness of tracing paper. These same thicknesses of the paper were then used to cover completely Petri dishes set up for conidial germination at different light intensities.

Set-ups that required dark conditions were either covered with a black cloth or placed in a cupboard, which was tightly closed, cutting out the entry of any light.

n. Germination of the conidia on surfaces of leaves of different R.W.C.

Epidermal strips were removed from inoculated sites of leaves of different R.W.C and placed in tiny drops of lactophenol cotton blue on glass slides with the inoculated surface uppermost. The epidermal strips were covered with cover slips and examined under the microscope. Percentage germination of each preparation was estimated.

o. Studies on Morphology of the Fungus.

Size of conidia.

The length and width of fully turgid conidia were measured. For cylindrical conidia the width measurement was taken at the mid-region of the conidium. For conical conidia, the width was taken at the base of the pointed end where the conidium was usually widest. A drop of water was placed on the slide by the spore print during measurement to maintain a humid atmosphere around the conidia and keep them turgid. Measurements were made with eye-piece graticule.

Length and form of conidiophores.

Square pieces of mildewed leaf, 5mm x 5mm, were cut and placed in a concentrated solution of chloral hydrate for 24 hours to clear. They were then stained in lactophenol cotton blue and mounted in plain lactophenol with the spore-bearing surface upwards. The number of branches was recorded and length of the main conidiophore measured using an eye-piece graticule.

Form and size of haustoria

The lower epidemis of the mildewed leaf was first removed to allow greater contact between the stain and the endotrophic hyphae, which were more crowded in the spongy mesophyll. Square pieces of the leaf, measuring 5mm x 5mm were then cut and placed in concentrated solution of chloral hydrate for 24 hours. They were then transferred to a mini Petri dish (6mm in diameter) containing lactophenol cotton blue, in which they could be kept until they were examined. They were examined under the microscope as temporary mounts in plain lactophenol. The spongy mesophyll side was always kept uppermost for ease of observation.

Diameter of haustoria were measured with an eye-piece graticule and camera lucida drawings were made of samples of haustorica of the different treatments.

p. Examination of stems of host for mycelium invasion.

The method used by Phillips and Hayman (1970) for clearing roots and staining vesicular arbuscular mycorrhizal fungi in root tissues was followed. According to Phillips and Hayman (op. cit.) the method has also proved successful for parasitic fungi.

Pieces of stem of infected host plants were fixed in FAA (13ml formalin, 5ml glacial acetic acid, 200ml 50% ethanol). Transverse sections of the fixed stems were heated at 90°C in 10% KOH for one hour to remove the host cytoplasm. The sections were then rinsed in distilled water and acidified with dilute HCl. They were finally stained by simmering for five minutes in cotton blue. The sections were transferred to plain lactophenol to remove the excess stain, and then mounted temporarily in plain lactophenol and observed under the microscope.

q. Methods of sterilization.

Conical flasks, pipettes and Petri dishes were soaked in potassium dichromate for 48 hours, washed several times under running tap and rinsed in de-ionized water. They were airdried and then sterilized in an oven at 160°C for six hours.

Glass slides and solid watch glass covers were washed with detergent, rinsed under running tap and stored in 95% methanol. They were flame-sterilized just before use.

Solid watch glasses previously washed with detergent and rinsed with distilled water were sterilized by rinsing with 70% Ethanol.

r. Photography

Photographs were taken to illustrate some aspects of the investigation with a 'Minolta x-19' camera and a Kodak film.

s. Experimental precautions.

1. Insects and mites were screened off by placing the potted plants in insect proof netting. Mites, especially, are known to feed on ectophytic mycelium.
2. During transplanting, the seedlings were carefully lifted from the plots with a ball of soil around the roots to keep their growing ends intact.
3. In removing the leaf-discs, a sharp cork borer was used to prevent sap exudation, which will introduce errors according to Slayter (1967).
4. In setting up spore germination tests using Van Tieghem cell, care was always taken to prevent the solution in the well from coming into contact with the spores.
5. Spore prints for germination tests had fairly evenly dispersed conidia and a uniform spore density of about 40 conidia per high power microscope field was used for the various tests.
6. In the germination tests the age of conidia employed was standardized by using conidia formed within 24 hours, prior to each test.

# III. EXPERIMENTAL DETAILS.

## A. Description of powdery mildew disease of *Galphimia glauca* caused by *Leveillula taurica*.

The epidemiology of a plant disease is literally the base-line information for studies on the host-parasite relationship. The nature of the powdery mildew disease of *G. glauca* caused by *Leveillula taurica* was, therefore, studied on plants growing in the ornamental garden in front of the Balme Library of the University. The studies were carried out on two separate occasions, in November 1996 and May 1997, which fell in the dry (harmattan) and rainy seasons, respectively.

Plants of different ages were inspected for infection. The leaves, flowers and green stems were closely examined using a hand lens for the earliest signs of infection. The later stages of the disease were observed with unaided eyes. Initial infection and progress of the disease and the symptoms at the various stages of infection are described in Section A of the Results and shown in Plates 1-3, while some of the quantitative data obtained are recorded in Table 3.

## B. Studies on Mycelial features and conidial apparatus of *L. taurica* strain infecting *G. glauca* plants.

Strains of *L. taurica* infecting other host plants in Ghana have been found to show differences especially in the dimensions of the conidia. The mycelium and asexual reproductive structures of the strain on *G. glauca* were, therefore, studied under the high power objective of the microscope to establish similarities and differences between it and the other strains. The conidia were examined without staining, while the mycelium in leaves cleared with chloral hydrate and conidiophores on the leaves were stained with lactophenol cotton blue. Observations made on the shape and size of the haustoria, conidiophore length, branching habit of the conidiophores, and conidial dimensions are recorded in Section B of the Results. Camera lucida drawings made of the hyphae and haustoria, and histogram of range of lengths of conidiophores are shown in Figs. 3 and 4, respectively. Data on the range of sizes of haustoria and conidia are shown in Tables 4 and 5, respectively. Lastly, data on the form of conidiophores are recorded in Table 6 and Fig 5.

**C. Studies on the germination of the conidia at different light intensities.**

Light affects germination of conidia of some fungal species. It was considered necessary, therefore, to find out the best, light condition for germination of the *L. taurica* conidia so that it would be adopted in subsequent conidial germination experiments.

Three series of experiments were conducted:

(a) Conidia were incubated in either dark or light of 76 lux intensity, at 100% RH, using the glass slide method.

(b) Two series of tests were next conducted to find the effect of light - condition during sporulation on subsequent germination of the conidia. In one series, conidia formed in dark were divided into two batches, one was incubated in dark and the other incubated in light of 76 lux intensity at 100% R.H: In the other series, conidia were formed in light, then divided into two batches. One batch was germinated at 100% R.H. in dark and the other at 100% R.H. in light of 76 lux intensity.

(c) Because the conidia germinated better in light of 76 lux in the two previous experiments, germination in light of five different intensities of 22, 32, 43, 54, and 76 lux was investigated.

The results of the various experiments appear in Section C of the Results and in Tables 7-9.

**D. Studies on the characteristics of conidia of L. taurica growing in leaves of G. glauca of different R.W.C's**

Several 4-leaf stage healthy young plants were transplanted into potting bags and placed on the eastern verandah of the Research Laboratory. They were watered daily for three days and then inoculated. The daily watering was continued for further seven days when diseased lesions were clearly observable and sporulating conidiophores could be detected with the aid of a hand lens.

The plants were divided into four batches of four plants each and were, thereafter, watered at different intervals:

| | |
|---|---|
| Batch 1: | once every day. |
| Batch 2: | once every 2 days. |
| Batch 3: | once every 3 days. |
| Batch 4: | once every 4 days. |

The watering was carried out for 10 days to ensure that the different R.W.C's had stabilized for each treatment. Samples of leaves of the different treatments were collected, with the sampling time falling between two waterings for each of treatments for Batches 2, 3 and 4. The following schedule for collection was used and the R.W.C's of the harvested leaves determined:

| | |
|---|---|
| Batch 1: | Leaves collected on the 4$^{th}$ day |
| Batch 2 | Leaves collected on the 4$^{th}$ day |
| Batch 3: | Leaves collected on the 8$^{th}$ day |
| Batch 4: | Leaves collected on the 8$^{th}$ day. |

The plants were then vigorously shaken on the same day, after the leaf samples had been collected, to dislodge all conidia present on conidiophores on the remaining leaves, and the watering regimes continued. New conidia were thus formed by mycelium dependent on leaves of a particular R.W.C. The conidia produced were collected onto clean glass slides at the following times from the centre of the lesions to ensure that they came from already existing conidiophores before the water content was reduced;

Conidia from Batch 1:collected on the $4^{th}$ day

Conidia from Batch 2:collected on the $4^{th}$ day

Conidia from Batch 3:collected on the $8^{th}$ day

Conidia from Batch 4:collected on the $8^{th}$ day.

The entire procedure is illustrated in the flow-chart in Fig. 1.

The following characteristics of the conidia of the different treatments were determined:

(a) length and width of the conidia.

(b) maximum length of germ tube supported by the endogenous substrate of the conidia.

The germ tubes were measured after 48 hours. Results of a preliminary test showed that there was very slight increase in germ tube length from 36 to 48 hours, and after 48 hours the germ tubes started to break up.

(c ) relative water content of the conidia, by estimating the rate of shrinking of the conidia kept at 0.0% R.H.

The results of these tests and the calculated R.W.C's of the leaves of the different watering regimes appear in Tables 10 - 13

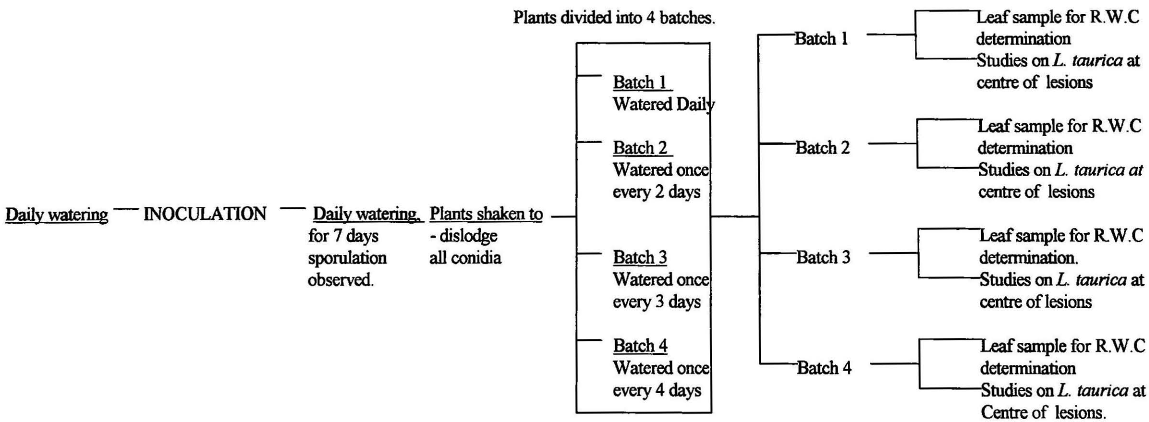


FIG. 1 Flow chart of procedure used to study the effect of adjusting the water content of mycelium of *L. taurica* on sporulation of the fungus.

**E. Studies on the size and form of conidiophores and size of conidia of mycelia of different moisture content induced by adjusting host leaves to different R.W.C.**

In the preceding experiment, the water content of the leaf was adjusted after the mycelium was well established and conidiophores had developed. It was, therefore, only the process of conidiation which was studied at different water content levels in the leaves. The treatment affected the conidia, which were formed in many ways. This experiment was set up to find to what extent the conidia would be affected if the mycelium also developed at lower levels of water content as well.

The illustration in Fig. 2 shows the steps, which were followed. The plants were watered daily for 3 days before inoculation and then watered daily again for 7 days when infection was well established. The plants were divided into four batches and Batches 1, 2, 3 and 4 were watered daily, once in 2 days, once in 3 days and once in 4 days, respectively, over 12 days. The outline of selected lesions on plants of the different R.W.C. was marked on the adaxial surfaces of the leaves with Mandarin (Indian) Ink using a camel hair brush. The watering treatment was continued for further 12 days. The mycelium beyond that margin was naturally formed under the particular R.W.C. of the leaf. For each treatment, the leaf was cut with a pair of fine scissors along the marked perimeter and the old lesion discarded. The mycelium, conidiophores and conidia lying beyond the perimeter were studied.

Conidia of mycelia in leaves of different R.W.C. and therefore also different water content were harvested on clean glass slides and their lengths and widths were measured. Camera lucida drawings of representative conidia are shown in Fig. 6 and the measurements made are presented in Table 14.

Pieces of the leaves of this region measuring 5mm x 5mm were next cut with scapel and cleared in chloral hydrate solution. They were then stained with lactophenol cotton blue and mounted in plain lactophenol for microscopic study of the conidiophores. The form of the conidiophores from leaves of different R.W.C.'s was studied and the lengths of unbranched conidiophores measured with an ocular graticule. The germination capacity of the conidia and maximum length of germ tubes were also determined using the glass slide method. The results are recorded in Table 15 and Fig. 8.

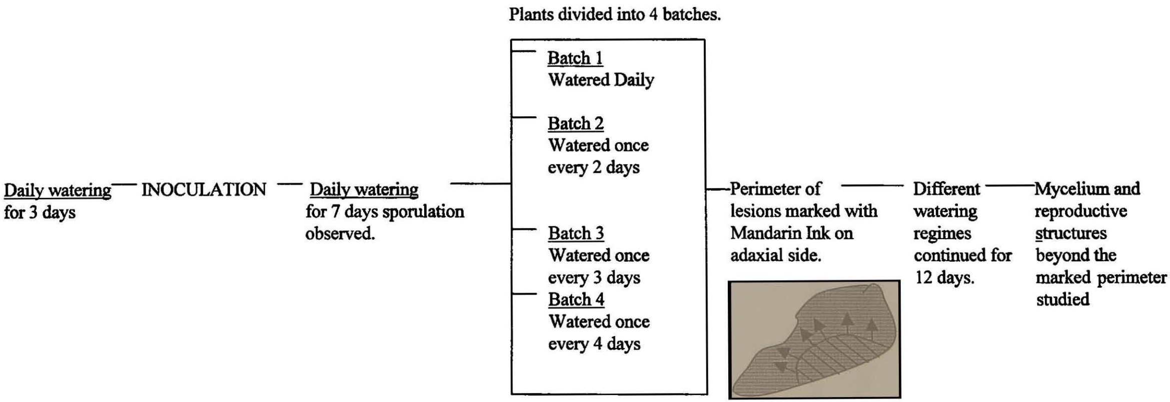


FIG. 2 Flow chart of procedure used to study development and sporulation of *L. taurica*.

**F. Studies on the level of endogenous substrates and germination capacity of *L. taurica* conidia formed by mycelium growing in mature leaves of different R.W.C.**

The next investigation examined the relative amount of endogenous substrates and the germination capacity of the conidia of different treatments given in Experiment E.

The same experimental procedure was followed and conidia from leaves of each of the different R.W.C. were germinated at 100% R.H. at $30^0$ C for 48 hours. As noted in Section D, the germ tubes broke up thereafter. The maximum lengths of the germ tubes were considered as indicative of the relative amounts of endogenous nutrients available in the conidia. The data obtained are shown in Table 16.

Several sets of conidia from leaves of each of the different R.W.C. were again collected on clean glass slides. Seven sets were prepared for each R.W.C. treatment and incubated at $30^0$C at 0.0, 16.9, 44.8, 62.5, 85.2, 92.8, and 100% R.H., respectively. In the experiment of Section D, the conidia formed on leaves of the different R.W.C. were germinated at 100% R.H. only. In that experiment the conidia of the different R.W.C's showed different percentage germination, which declined with decreasing R.W.C. This experiment sought to find out the combined effect of decreasing percentage relative humidity of the atmosphere of the germination chamber and R.W.C. of the leaves on which they were formed. At the end of the incubation period, the percentage germination of the conidia of each was determined and 20 germ tubes of each treatment measured with the ocular graticule. The results are shown in Table 17 and Fig. 9.

**G. Studies on the size of haustoria of *L. taurica* mycelia of different Relative Water Content induced by adjusting host leaves to different R.W.C.**

Using the same treatments of Experiment E, the lower epidermis of regions of the leaf between the two perimeters was removed with a pair of fine forceps and the lamina cut into 5mm X 5mm squares. The pieces were put in a concentrated chloral hydrate solution overnight to clear the chlorophyll, and then stained with lactophenol cotton blue. They were finally mounted in plain lactophenol with the exposed spongy mesophyll tissue uppermost.

The preparations were examined under the H.P. of the microscope to study the mycelia and the camera lucida drawings in Fig. 10 were made of representative haustoria of each treatment. The diameters of the haustoria were then measured and the mean of 20 haustorial diameters for each treatment calculated. The results obtained are shown in Table 18.

**H. Studies on germination of conidia produced by *L. taurica* mycelia of different moisture content on uninfected leaves of *G. glauca* of different R.W.C.**

Leaves, stems and roots produce exudates which influence micro-organisms on their surfaces and in their immediate vicinities. Exudation involves both secretion requiring metabolic energy and passive diffusion. The physiological status of the organ is known to affect exudation. This experiment was carried out to find out whether conidia of *L. taurica* germinating on leaves of *G. glauca* of different R.W.C's which would naturally affect the leaf physiology, would behave differently. Leaves of different R.W.C's were surface - inoculated with conidia formed by mycelia growing in *G. glauca* leaves also of different R.W.C's in all possible combinations, using plants watered daily, once in 3 days, and once in 4 days.

Lesions with actively sporulating conidiophores were gently adpressed to the abaxial surface of the fresh leaves. The inoculated leaves were cut off after 24 hours for examination. Epidermal strips were removed form the inoculated sites and placed in tiny drops of lactophenol cotton blue on glass slides with the inoculated surface uppermost. The epidermal strips were covered with cover slips and examined under the microscope. Percentage germination of each preparation was estimated and 20 randomly selected germ tubes in each were measured. The data obtained are shown in Table 19. Camera lucida drawings of Fig. 11 were also made of samples of the germinated conidia to illustrate the course of growth of the germ tubes on the leaves.

**I. Studies on infection rate of *G. glauca* leaves of different R.W.C. surface inoculated with *L. taurica* conidia.**

Since the results of the last experiment showed a clear effect of R.W.C. of the leaves on germination of the conidia, it was presumed that infection rate would also be affected as well. This experiment was set up to verify this supposition.

The experiment used as inoculum conidia of mycelia in *G. glauca* leaves of plants watered daily, once in 3 days, and, once in 4 days. The *G. glauca* plants to be inoculated were also subjected to these three watering regimes. The inoculation was again carried out using all combinations of the three watering treatments.

Leaves of the healthy plants were inoculated on the abaxial surface as described in Section H, ensuring that approximately the same amount of inoculum was used in all by using identical discs of the sporulating lesion as the conidial source. The inoculated sites were marked on the adaxial surface with Mandarin ink. The inoculated surfaces were then examined at noon each day with a hand lens for signs of developing conidiophores. The dates conidophores appeared in the different inoculations were noted accordingly. The results are shown in Table 20.

**J Studies on the extent of growth of *L. taurica* mycelium in the host plant.**

Since the petioles of the leaves are very short, there was the possibility that the mycelium might grow into the stems, from where they might invade new leaves. The stem was, therefore, extensively examined for the presence of mycelia, using the method described under General Methods, Section l.

Observation of stems of infected plants was done in the field with a hand lens and later closer inspection was carried out in the laboratory. Ten stem pieces each from five randomly selected infected plants were used for the study. For each piece, transverse sections were cut from the region immediately below the growing tip, from the internodes and nodes.

# IV. RESULTS

## A. DESCRIPTION OF POWDERY MILDEW DISEASE OF *GALPHIMIA GLAUCA* CAUSED BY *LEVEILLULA TAURICA*

Powdery mildew disease of *Galphimia glauca* follows the observed course of infection of *L. taurica* in pepper and other host plants, but with some variation. Infection was always detected first on older leaves of the plant. Plates 1 and 2 show that the disease appears as conspicuous white lesions which initially occur randomly only on the lower surface of the leaves. The whitish appearance of the lesions is due to the numerous erect conidiophores and conidia of the pathogen easily detectable with a hand lens. As the disease progresses, the whitish lesions could be observed not only on the lower surface of the leaf, but on the upper surface as well. Spreading lesions on a leaf eventually merge and the entire leaf surface may be covered by the white powdery mass.

Plate 3 shows another aspect of the disease. During the later stages of the infection, the leaf becomes chlorotic with occasional patches of green tissue forming what is termed 'green islands' in plant pathology (Kiraly, Hammady and Pozsar, 1967). Mildewed leaves are prone to premature abscission, causing infected plants to become partially defoliated. Infected plants, however, did not die of the disease, even with as high as 73.0 percent of foliage infection. The results of a survey of the plants in the plot presented in Table 3 showed that more than half the number of leaves on the plant could be infected. No lesions were observed on petioles, green stems and floral parts of the plant.

The disease is seasonal. Infection was heaviest in the rainy season and almost non-existent in the dry season.

Table 3.

**Extent of *Leveillula taurica* infection of randomly selected mature *Galphimia glauca* plants in flower beds in front of Balme Library, in May, 1997.**

| Plant | Total Number of leaves on plant. | Number of leaves with *L. taurica* lesions. | Percentage of leaves with *L.taurica* lesions. |
|---|---|---|---|
| A | 203 | 147 | 72.4 |
| B | 456 | 333 | 73.0 |
| C | 247 | 171 | 69.4 |
| D | 501 | 246 | 52.7 |
| E | 232 | 121 | 52.2 |
| Mean | 327 | 207 | 63.3 |

Plate 1. Photograph of branches of *Galphimia glauca* showing lesions of *Leveillula taurica* on the abaxial surfaces of the leaves (x1).

Plate 2. Photograph of abaxial surfaces of leaves of *Galphimia glauca* almost completely covered with lesions of *Leveillula taurica* at advanced stage of infection. Leaf at extreme left is uninfected (x1).

Plate 3. Photograph of young plants of *Galphimia glauca* infected with *Leveillula taurica* showing development of 'green islands' in the prematurely chlorotic infected leaves (x1).

## B. STUDIES ON MYCELIAL FEATURES AND CONIDIAL APPARATUS OF *L. TAURICA* STRAIN INFECTING *G. GLAUCA* PLANTS.

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### i Size of *L. taurica* haustoria.

*L. taurica* forms globose haustoria in the mesophyll cells of the leaves of the host, *Galphimia glauca.* Fig. 3 and Appendix A indicate the range of diameters of the haustoria. They ranged from 6.7 μm in diameter to 23.3 μm, with a mean of 14.2 μm. Table 4 shows that the values of relative abundance (Percentage Frequency) of the different class-diameters were the same.

### ii. Size of *L. taurica* conidia.

*L. taurica* forms both conical and cylindrical conidia. Ayesu-Offei (1966) reported that there was a highly significant difference between the mean lengths of the two types of conidia of *L. taurica* formed on pepper plants. Palti (1988) also reported that the size of conidia of *L. taurica* may vary appreciably even when derived from one and the same host species.

In the present study the data in Table 5 show that the cylindrical conidia were generally longer and broader than the conical conidia and the mean lengths of the cylindrical and conical conidia were 63.8μm and 55.8 μm, respectively, while mean widths of the two types of conidia were 12.7μm and 11.6μm, respectively. While with both conidia, the peak relative abundance fell in the 12.0 - 15.9 μm class-width, the peak was different for conidial lengths. It was 56.0 – 65.9 μm class-length for the cylindrical conidia and 46.0 - 55.9 μm class-length for the conical conidia.

iii. <u>Forms of *L. taurica* conidiophores.</u>

Conidiophores of *L. taurica* may be branched or unbranched. The lengths of unbranched conidiophores formed on naturally infected *G. glauca* plants varied extensively. A conidiophore which has attained maximum length has a blunt rounded apex as the initial pointed end has formed the first and conical conidium. The lengths ranged from 40.0 to 189.8 μm with a mean length of 101.4 μm and 37.0 percent of the conidiophores were unbranched. Fig. 4 shows the percentage frequencies of the conidiophores in the different class-lengths. A high percentage (60 percent) of the conidiophores fell within the 50.1 - 80.0 μm and 80.1 - 110.0 μm class-lengths.

Table 6 contains the relative abundance of the different branched conidiophores. Slightly above 60 percent of the conidiophores had only two branches. The maximum number of branches observed was four. Fig. 5 shows that a branch may be longer than the apparent main stem and so lengths were not measured as the longest values may not relate to the same feature.

Table 4.

**Relative abundance (Percentage Frequency) of different - sized haustoria of *Leveillula taurica* formed in mesophyll cells of leaves of *Galphimia glauca*.**

| Class - Diameter (μm) | Percentage Frequency |
|---|---|
| 5.0 - 9.9 | 25 |
| 10.0 - 14.9 | 25 |
| 15.0 - 19.9 | 25 |
| 20.0 - 24.9 | 25 |
| Mean Haustorial Diameter: | 14.2 ± 0.11 μm |

(Data for Percentage Frequency calculation in Appendix A).

Table 5

**Frequency distribution of lengths and widths of conidia of *Leveillula taurica*.**

| Class-Length (μm) | Conical Conidium | Cylindrical Conidium |
|---|---|---|
| 36.0 - 45.9 | 2 | 1 |
| 46.0 - 55.9 | 49 | 34 |
| 56.0 - 65.9 | 39 | 40 |
| 66.0 - 75.9 | 10 | 23 |
| 76.0 - 85.9 | 0 | 2 |
| Mean length ± S.E. | 55.8 ± 0.03μm | 63.8 ± 0.12μm |
| Class-width | | |
| 4.0 - 07.9 | 14 | 1 |
| 8.0 - 11.9 | 34 | 33 |
| 12.0 - 15.9 | 41 | 51 |
| 16.0 - 19.9 | 11 | 14 |
| 20.0 - 23.9 | 0 | 1 |
| Mean width ± S.E. | 11.6 ± 0.00μm | 12.7 ± 0.10μm |

Table 6.

**Relative abundance (Percentage Frequency) of conidiophores of *Leveillula taurica* with two, three or four branches observed in 20 high power microscope fields of lesions on *Galphimia glauca* leaves.**

| Number of branches on a conidiophore | Number of conidiophores (out of a total of 84) | Percentage Frequency |
|---|---|---|
| 2 | 52 | 61.9 |
| 3 | 12 | 14.3 |
| 4 | 20 | 23.8 |

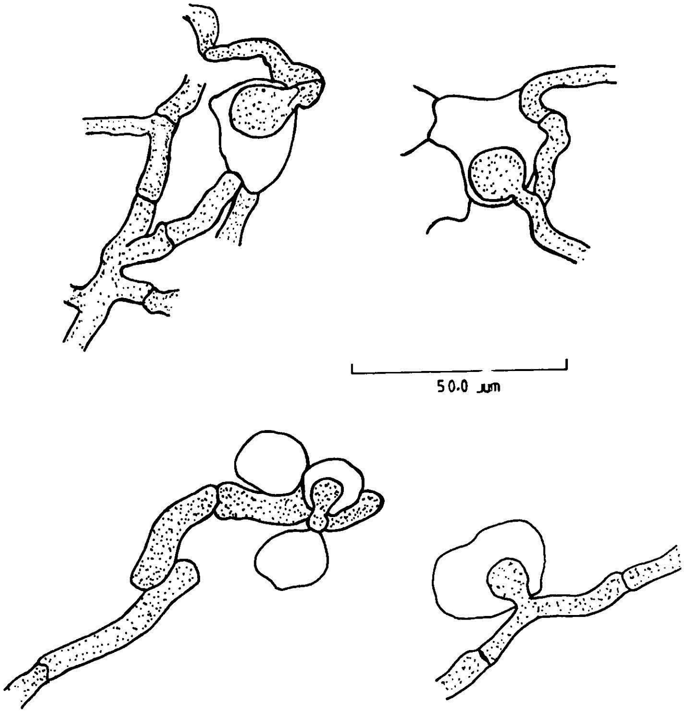


Fig. 3 Camera lucida drawings of samples of haustoria of *Leveillula taurica* formed in mesophyll cells of leaves of *Galphimia glauca* showing differences in haustorial sizes.

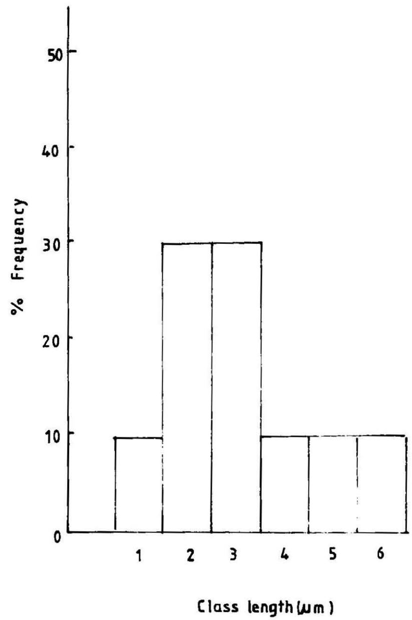


Fig. 4 Relative abundance (Percentage Freqency) of unbranched Leveillula taurica conidiophores in different class-lengths formed by mycelium in Galphimia glauca leaves

1. 20.1 - 50.0 µm
2. 50.1 - 80.0 µm
3. 80 1 - 110 0 µm
4. 110.1 - 140.0 µm
5. 140·1 - 170.0 µm
6. 170·1 - 200 0 µm

(Data for histogram in Appendix B)

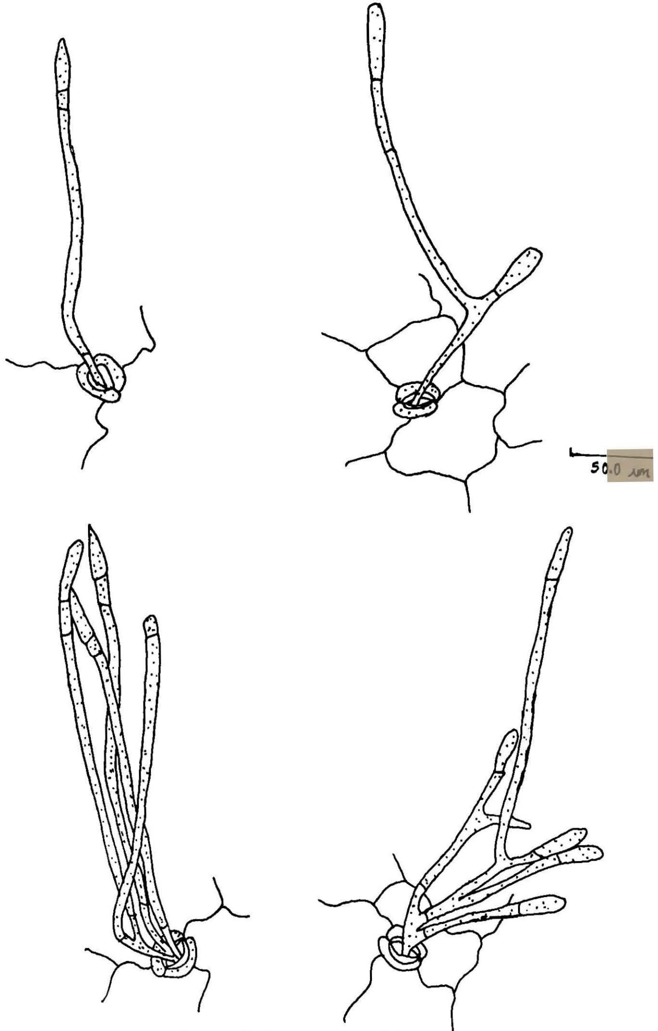


Fig. 5 Camera lucida drawings of forms of conidiophores of Leveillula taurica formed on Galphimia glauca leaves

## C. STUDIES ON THE GERMINATION OF THE CONIDIA AT DIFFERENT LIGHT INTENSITIES.

### i. Comparative germination of the conidia in light and dark.

Table 7 shows the results of germination tests carried out in which the conidia were either germinated in dark or in light of intensity of 76 lux. Germination of conical and cylindrical conidia was determined separately.

In both light and dark, conical conidia germinated slightly better than cylindrical conidia. For each type of conidium, final germination after 48 hours in light of 76 lux was slightly better than in dark. Light could, however, be regarded as superior to dark when germination in shorter incubation periods of 12, 24, and 36 hours is considered. Thus, for both types of conidia percentage germination in light after 12 and 24 hours was more than two-fold than in dark. Similarly, the germ tubes formed in light were far longer than those formed in dark after 48 hours. The mean lengths of the 'light' germ tubes were 100.9 and 102.2 μm compared to 65.1 and 76.9 μm of the 'dark' germ tubes. It would be seen that the mean germ tube lengths of the two types of conidia were close under the same condition of incubation.

### ii. Germination in light of different intensities.

The results of the experiments on germination of the conidia under different intensities of light used two facts which emerged from the previous experiment. First, the conical and cylindrical conidia germinated to almost the same degree and were consequently put together in the determination of both conidial percentage germination and mean germ tube lengths. Secondly, since an incubation period of either 12 or 24 hours showed clearly the comparative effects of light and dark, an incubation period of 24 hours was used. The results tabulated in Table 8 could be summarised as follows:

1. Percentage germination in light of 22-76 lux was higher than in dark.
2. Mean germ tube lengths were greater in light of 22-76 lux than in dark.
3. Values of percentage germination in light of 22 and 32 lux were close and clearly far lower (21.2 and 22.7 percent, respectively) than values of percentage germination at 54 and 76 lux which were also close (34.2 and 36.9 percent, respectively). The effect of light intensity of 43 lux was median between the two extremes.
4. Mean germ tube length was apparently independent of light intensity within the range of 22 to 76 lux.

iii. Formation and germination of conidia in different light conditions.

Table 9 shows the results obtained when conidia formed in light of 76 lux were germinated in either light of 76 lux or dark and when the same germination conditions were used for conidia formed in dark. Conidia formed in light and germinated in light showed the highest percentage germination of 20.8 percent and the longest mean germ tube length of 69.1 μm. At the other extreme, conidia formed in dark and germinated in dark showed the least percentage germination of 11.4 percent and the shortest mean germ tube length of 43.4 μm. Results of a combination of the two treatments were intermediate.

Table 7.

**Germination of conidia of *Leveillula taurica* formed on leaves of *Galphimia glauca* at 100% R.H. at 30$^0$C for 48 hours in light and dark.**

| Light Condition and Type of Conidium | Percentage Germination after indicated hours of incubation. | | | | Mean Germ Tube Length (µm) after 48 Hours |
|---|---|---|---|---|---|
| | 12 | 24 | 36 | 48 | |
| Incubation in Light (76 lux) | | | | | |
| Conical | 28.0 | 29.7 | 38.9 | 63.7 | 102.2 ± 0.12 |
| Cylindrical | 25.0 | 25.5 | 39.3 | 52.8 | 100.9 ± 0.10 |
| Incubation in Dark | | | | | |
| Conical | 7.1 | 13.6 | 27.5 | 56.8 | 76.9 ± 0.06 |
| Cylindrical | 9.3 | 10.0 | 28.0 | 49.8 | 65.1 ± 0.12 |

Table 8

**Germination of conidia of *Leveillula taurica* formed on leaves of *Galphimia glauca* at 100% R.H. at 30$^0$C for 24 hours in light of different intensities.**

| Light intensity (Lux) | Percentage Germination | Mean Germ Tube Length (μm) |
|---|---|---|
| 76 | 36.9 | 61.2 ± 0.20 |
| 54 | 34.2 | 74.4 ± 0.15 |
| 43 | 26.8 | 66.7 ± 0.12 |
| 32 | 22.7 | 60.2 ± 0.03 |
| 22 | 21.2 | 70.5 ± 0.20 |
| 0 | 16.1 | 44.1 ± 0.04 |

Table 9

**Germination of conidia of *Leveillula taurica* formed on leaves of *Galphimia glauca* in either 24 hours continuous light of 76 lux or continuous darkness and incubated under different light conditions at 100% R.H. at 30$^0$C for 24 hours.**

| Light condition during conidium formation | Light condition during conidium germination | Percentage Germination | Mean Germ Tube Length (μm) |
|---|---|---|---|
| Light (76 lux) | Light (76 lux) | 20.8 | 69.1 ± 0.05 |
| | Dark | 18.7 | 53.5 ± 0.22 |
| Dark | Light (76 lux) | 15.7 | 52.3 ± 0.15 |
| | Dark | 11.4 | 43.4 ± 0.12 |

## D. STUDIES ON THE CHARACTERISTICS OF CONIDIA OF *L. TAURICA* GROWING IN LEAVES OF *G. GLAUCA* OF DIFFERENT RELATIVE WATER CONTENT (R.W.C).

### i. Effect of R.W.C. of leaves on size of conidia.

R.W.C. of leaves established in the different *G. glauca* plants by watering at different frequencies are shown in Table 10. As might be expected, plants watered daily had the highest R.W.C. of 80.6 percent, while leaves of plants watered once in 4 days had the lowest R.W.C. of 66.7 percent.

The data in Table 11 and Fig. 6 show that the sizes of the conidia decreased with increasing water stress. Conidia formed on leaves of 80.6 percent R.W.C. were longest (55.7 and 56.8 μm) and those formed on leaves of 66.7 percent R.W.C. were shortest (50.9 and 51.8 μm). The widths of the conidia followed the same trend, with the conical and cylindrical conidia from leaves of 80.6 percent R.W.C showing the respective mean widths of 14.0 and 13.2 μm and those from leaves of 66.7 percent R.W.C, the respective mean widths of 12.4 and 10.1 μm.

### ii. Effect of R.W.C. of leaves on germination capacity of conidia formed on them.

The data in Table 12 showed that both percentage germination and germ tube length of *L. taurica* conidia were affected by R.W.C. of the host leaves on which they were formed. Generally, percentage germination was related to the R.W.C. The higher the water content the greater the percentage germination of conidia formed on the leaves. The lengths of the germ tubes also showed the same trend. Therefore, a percentage germination of 48.9 percent was attained by conidia of leaves of 80.6

Table 10.

**Relative Water Content established in leaves of different *Galphimia glauca* plants by watering the plants at different frequencies with same amount of water.**

| Frequency of Watering | Mean Relative Water Content (%) |
|---|---|
| Daily | 80.6 |
| Once in 2 days | 78.4 |
| Once in 3 days | 74.4 |
| Once in 4 days | 66.7 |

Table 11

Relative abundance (Percentage Frequency) in the different class-lengths and class-widths of conidia of *Leveillula taurica* formed on leaves of *Galphimia glauca* of different Relative Water Content (RWC).

| R.W.C of Leaves (%) | Conical Conidia: Class-length (µm) 25.0-29.9 | 30.0-39.9 | 40.0-49.9 | 50.0-59.9 | 60.0-69.9 | Mean Length ± S.E. (µm) | Cylindrical Conidia: Class-length (µm) 25.0-29.9 | 30.0-39.9 | 40.0-49.9 | 50.0-59.9 | 60.0-69.9 | Mean Length ± S.E. (µm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 80.6 | 0 | 0 | 0 | 92 | 8 | 55.7 ± 0.02d* | 0 | 0 | 0 | 86 | 14 | 56.8 ± 0.00c |
| 78.4 | 0 | 0 | 12 | 84 | 4 | 53.4 ± 0.04c | 0 | 0 | 10 | 86 | 4 | 55.3 ± 0.00b |
| 74.4 | 2 | 4 | 16 | 72 | 6 | 51.4 ± 0.03a | 0 | 0 | 10 | 82 | 8 | 56.0 ± 0.15b |
| 66.7 | 0 | 2 | 20 | 70 | 8 | 51.8 ± 0.03b | 0 | 0 | 26 | 72 | 2 | 50.9 ± 0.00a |

| R.W.C of Leaves (%) | Conical Conidia: Class-width (µm) 6.0-8.9 | 9.0-11.9 | 12.0-14.9 | 15.0-17.9 | 18.0-20.9 | Mean width ± S.E. (µm) | Cylindrical Conidia: Class-width (µm) 6.0-8.9 | 9.0-11.9 | 12.0-14.9 | 15.0-17.9 | 18.0-20.9 | Mean width ± S.E. (µm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 80.6 | 0 | 4 | 60 | 36 | 0 | 14.0 ± 0.39 c | 0 | 16 | 72 | 12 | 0 | 13.2 ± 0.02 c |
| 78.4 | 0 | 20 | 76 | 4 | 0 | 12.8 ± 0.02 b | 4 | 64 | 32 | 0 | 0 | 10.9 ± 0.00 b. |
| 74.4 | 0 | 30 | 66 | 4 | 0 | 12.4 ± 0.05 a | 18 | 56 | 26 | 0 | 0 | 10.3 ± 0.04 a |
| 66.7 | 0 | 36 | 56 | 8 | 0 | 12.4 ± 0.02 a | 24 | 50 | 0 | 0 | 0 | 10.1 ± 0.03 a |

* By calculated confidence Limits, figures bearing the same letters are significantly not different at 95% level.

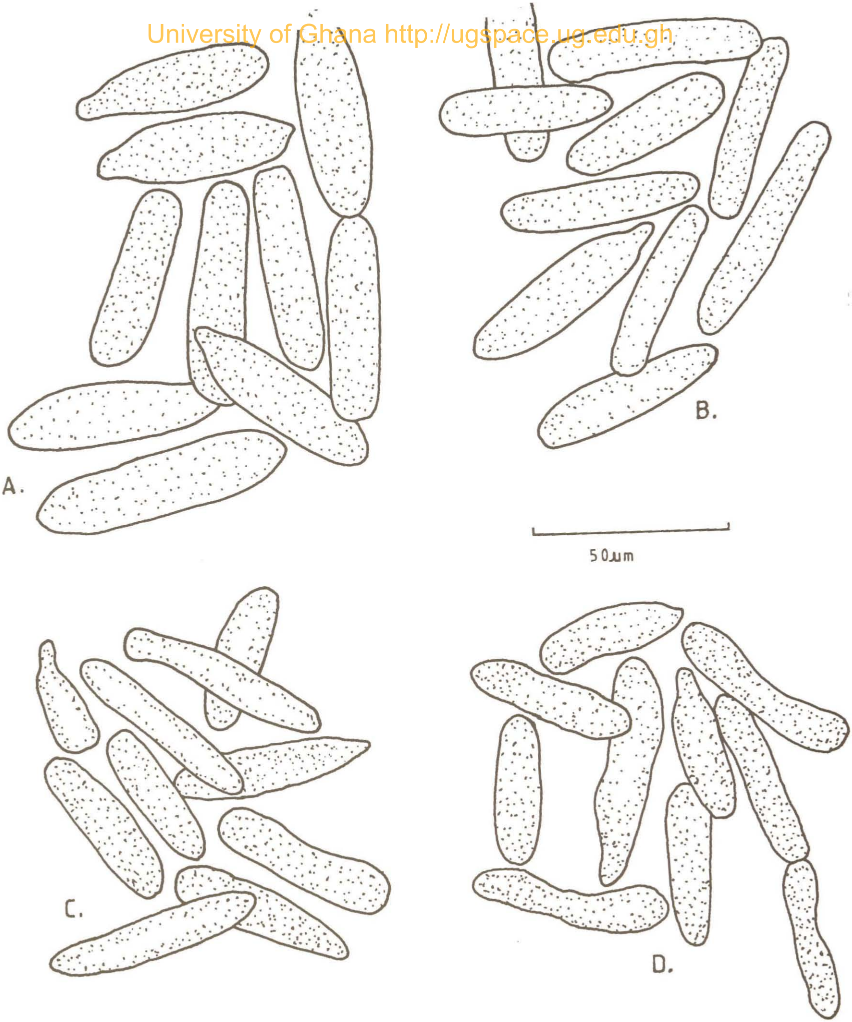


Fig.6 Camera lucida drawings of conidia formed by Leveillula taurica on the leaves of Galphimia glauca of different R.W.C.s

A – 80.6 %

B – 78.4 %

C – 74,4 %

D – 66,7 %

Table 12.

**Germination and growth of germ tubes in 48 hours at 30$^0$C of conidia of *Leveillula taurica* formed after adjustment of the R.W.C. of leaves of the host, *Galphimia glauca.***

| R.W.C. of leaf % | Percentage Germination | Mean Germ Tube Length ± S.E. (μm) |
|---|---|---|
| 80.6 | 48.9 | 188.5 ± 0.11 |
| 78.4 | 31.5 | 179.4 ± 0.05 |
| 74.4 | 34.6 | 94.5 ± 0.21 |
| 66.7 | 12.2 | 72.6 ± 0.03 |

percent R.W.C. and only 12.2 percent by conidia of leaves of 66.7 percent R.W.C. Mean germ tube lengths ranged from the lowest 72.6 to the highest 188.5 μm for conidia from leaves of 66.7 and 80.6 percent R.W.C. respectively.

iii. <u>Relationship between water content of the conidia and the R.W.C. of the leaes on which they were formed.</u>

The data in Table 13 provided an indirect information on the amount of water in conidia formed on leaves of different R.W.C. In an atmosphere of 0.0% R.H. all the conidia formed on leaves of 66.7 percent R.W.C. were shrunken within 50 minutes, compared to 62.4, 20.0 and only 7.6 percent of conidia formed on leaves of 74.4, 78.4 and 80.6 percent R.W.C. respectively. After 100 minutes, the percentage of conidia from leaves of these three R.W.C. shrunken was 84.0, 68.6 and only 16.2, respectively. It would require more than 180 minutes form all conidia of these three categories to shrink. The graph in Fig. 7 depicts the comparative rates of shrinkage of conidia of the four categories.

Table 13

**Percentage of conidia of *Leveillula taurica* formed after adjustment of the R.W.C of leaves of the host, *Galphimia glauca*, which shrank during storage in an atmosphere of 0.0% R.H. at 30$^0$C over 180 minutes.**

(Each value of percentage shrunken based on a total of 80-125 conidia.)

| Period of Storage (Minutes) | Percentage of conidia from leaves of indicated R.W.C (%) shrunken. | | | |
|---|---|---|---|---|
| | 80.6 | 78.4 | 74.4 | 66.7 |
| 0 | 2.9 | 3.6 | 12.5 | 8.2 |
| 10 | 2.9 | 6.3 | 20.8 | 71.4 |
| 20 | 3.8 | 9.4 | 37.9 | 73.5 |
| 30 | 6.7 | 13.5 | 40.7 | 87.5 |
| 40 | 7.6 | 15.4 | 53.1 | 90.7 |
| 50 | 7.6 | 20.0 | 62.4 | 100.0 |
| 60 | 9.5 | 36.8 | 65.3 | - |
| 70 | 10.5 | 41.9 | 69.4 | - |
| 80 | 13.3 | 62.9 | 83.1 | - |
| 90 | 13.3 | 65.8 | 84.0 | - |
| 100 | 16.2 | 68.6 | 84.0 | - |
| 110 | 17.1 | 77.1 | 87.2 | - |
| 120 | 42.6 | 77.1 | 89.5 | - |
| 130 | 75.9 | 80.0 | 93.5 | - |
| 140 | 79.3 | 82.9 | 92.1 | - |
| 150 | 81.6 | 82.9 | 94.7 | - |
| 160 | 81.6 | 83.3 | 94.2 | - |
| 170 | 87.0 | 85.7 | 97.1 | - |
| 180 | 87.0 | 90.0 | 97.1 | - |

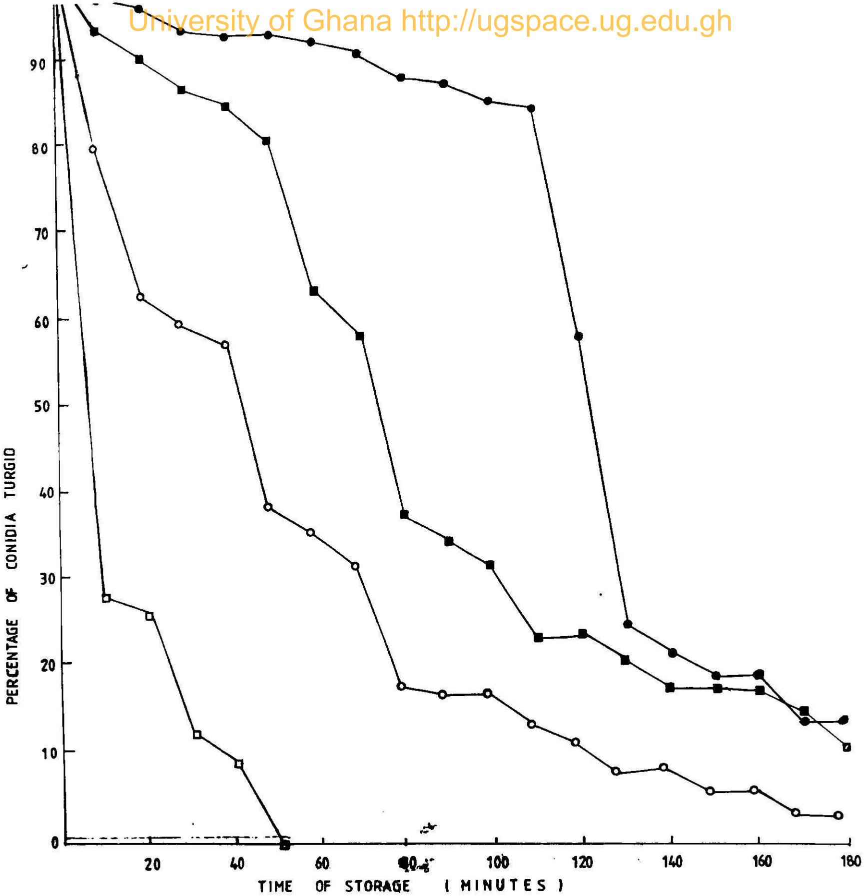


Fig. 7 Comparative rate of loss of water of conidia of <u>Leveillula taurica</u> formed after adjustment of the R.W.C. ( 66·7, □; 74·4, ○ ; 78·4, ■; and 80.6, ●; ) of leaves of the host <u>Galphimia glauca</u> on exposure to an atmosphere of 0 % R.H. at 30°C

(Values for graph calculated from data in Table 13.)

## E. STUDIES ON THE SIZE AND FORM OF CONIDIOPHORES AND SIZE OF CONIDIA OF MYCELIA OF DIFFERENT MOISTURE CONTENT INDUCED BY ADJUSTING HOST LEAVES TO DIFFERENT R.W.C.'S.

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When conidia formed by mycelia in *G. glauca* leaves of the different R.W.C. were measured, different ranges of dimensions were observed for the different categories of conidia:

i. Conical conidia from leaves of 80.6 percent R.W.C. ranged from 40.0 to 69.9 μm in length, and from 10.0 to 16.7 μm in width. Cylindrical conidia ranged from 50.0 to 66.6 μm in length and from 10.0 to 16.7 μm in width.

ii. Conical conidia from leaves of 78.4 percent R.W.C. ranged from 43.3 to 66.6 μm in length, and from 10.0 to 16.7 μm in width. Cylindrical conidia ranged from 46.6 to 66.6. μm in length and from 6.7 to 13.3 μm in width.

iii. Conical conidia from leaves of 74.4 percent R.W.C. ranged from 26.6 to 66.6 μm in length, and from 10.0 to 16.7 μm in width. Cylindrical conidia ranged from 30.0 to 66.6 μm in length, and from 6.7 to 13.3 μm in width.

iv. Conical conidia from leaves of 66.7 percent R.W.C. ranged from 33.3 to 63.3 μm in length, and from 10.0 to 16.7 μm in width. Cylindrical conidia ranged from 43.3 to 63.3 μm in length, and from 6.7 to 13.3 μm in width.

The relative abundance of the conidia of the different categories in different class-lengths and class-widths is shown in Table 14. The calculated mean lengths and widths showed decreasing sizes as the Relative Water Content of the host leaves fell from 80.6 to 66.7 percent R.W.C. Related to this, conidia of leaves of 80.6 percent R.W.C. generally showed a higher percentage frequency in the higher 50.0 - 59.9 μm and 60.0 - 69.9 μm class-lengths put together than conidia of the other three categories. This also applied to the widths of the conidia. Greater percentage of the conidia from leaves of 80.6 percent R.W.C. fell in the two highest class-widths of 12.0-14.9 and 15.0-17.9 μm than conidia from leaves of the lower percent R.W.C. of 66.7, 74.4 and 78.4 percent R.W.C.

The present study also demonstrated that the R.W.C. of the host leaves affected conidiophore length, and branching. Conidiophore length decreased with decreasing percent R.W.C. The mean lengths of 80 unbranched conidiophores in each case were 180.4, 142.2, 123.4 and 101.7 μm for conidiophores on leaves of 80.6, 78.4, 74.4 and 66.7 percent R.W.C., respectively. Fig.8 is a histogram of the relative abundance of the conidiophores in the different class-lengths. As high as 60 percent of the conidiophores of leaves of 80.6 percent R.W.C. fell in the highest class-length (170.1-200.0 μm) compared to 5-25 percent for conidiophores of leaves with lower R.W.C. (66.7, 74.4 and 78.4 percent R.W.C.)

Higher R.W.C. encouraged branching. Thus, 48.6, 33.3, 20.0 and 7.7 percent of conidiophores of leaves of 80.6, 78.4, 74.4 and 66.7 percent R.W.C., respectively, had 3 to 4 branches. The results are shown in Table 15.

Table 14

Relative abundance (Percentage Frequency) in the different class-lengths and class-widths of conidia of *Leveillula taurica* formed on leaves of *Galphimia glauca* of different Relative Water Content (RWC).

| R.W.C of Leaves (%) | Conical Conidia: Class-length (μm) 25.0-29.9 | 30.0-39.9 | 40.0-49.9 | 50.0-59.9 | 60.0-69.9 | Mean Length ± S.E. (μm) | Cylindrical Conidia: Class-length (μm) 25.0-29.9 | 30.0-39.9 | 40.0-49.9 | 50.0-59.9 | 60.0-69.9 | Mean Length ± S.E. (μm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 80.6 | 0 | 0 | 6 | 90 | 4 | 54.8 ± 0.15d* | 0 | 0 | 6 | 88 | 6 | 55.6 ± 0.22c |
| 78.4 | 0 | 0 | 18 | 80 | 2 | 52.4 ± 0.03c | 0 | 0 | 4 | 84 | 12 | 55.7 ± 0.01b |
| 74.4 | 0 | 0 | 24 | 74 | 2 | 51.4 ± 0.03b | 0 | 10 | 20 | 68 | 2 | 50.6 ± 0.04a |
| 66.7 | 0 | 12 | 20 | 68 | 0 | 49.4 ± 0.47a | 0 | 2 | 16 | 72 | 10 | 51.5 ± 0.01b |

| R.W.C of leaves (%) | Conical Conidia: Class-width (μm) 6.0-8.9 | 9.0-11.9 | 12.0-14.9 | 15.0-17.9 | 18.0-20.9 | Mean width (μm) | Cylindrical Conidia: Class-width (μm) 6.0-8.9 | 9.0-11.9 | 12.0-14.9 | 15.0-17.9 | 18.0-20.9 | Mean ± S.E. (μm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 80.6 | 0 | 12 | 66 | 20 | 2 | 13.7 ± 0.02c | 0 | 22 | 78 | 0 | 0 | 12.6 ± 0.03a |
| 78.4 | 0 | 26 | 60 | 14 | 0 | 12.9 ± 0.02b | 2 | 32 | 64 | 2 | 0 | 12.2 ± 0.02a. |
| 74.4 | 0 | 14 | 74 | 12 | 0 | 13.2 ± 0.05c | 18 | 20 | 70 | 0 | 0 | 12.2 ± 0.22a |
| 66.7 | 4 | 22 | 68 | 6 | 0 | 12.5 ± 0.01a | 16 | 42 | 36 | 6 | 0 | 12.4 ± 0.54a |

* By calculated confidence Limits, figures bearing the same letters are significantly not different at 95% level.

Table 15

**Relative abundance (Percentage Frequency) of the forms of conidiphores of *Leveillula taurica* formed on mature leaves of *Galphimia glauca* of different R.W.C.**

| Leaf R.W.C (%) | Total Number of Conidiophores Observed. | Branched Conidiophores Number | Branched Conidiophores % | Number of branches on main Conidiophores | Percentage Frequency |
|---|---|---|---|---|---|
| 80.6 | 296 | 240 | 81.1 | 1 | 14.1 |
| | | | | 2 | 37.3 |
| | | | | 3-4 | 48.6 |
| 78.4 | 220 | 156 | 70.9 | 1 | 15.0 |
| | | | | 2 | 51.7 |
| | | | | 3-4 | 33.3 |
| 74.4 | 140 | 104 | 74.3 | 1 | 55.0 |
| | | | | 2 | 25.0 |
| | | | | 3-4 | 20.0 |
| 66.7 | 152 | 80 | 52.6 | 1 | 76.9 |
| | | | | 2 | 15.4 |
| | | | | 3-4 | 7.7 |

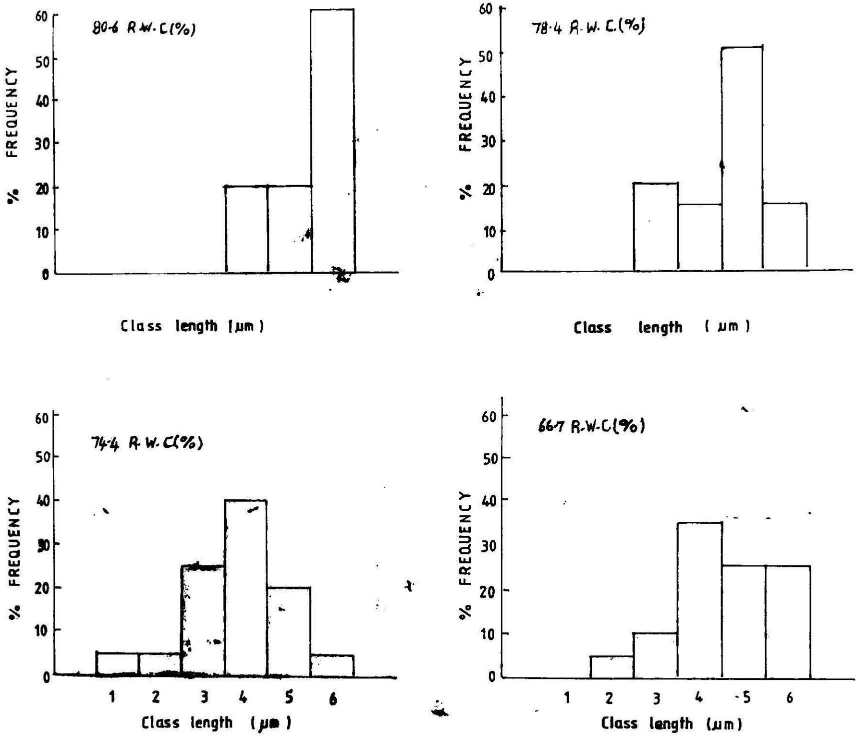


Fig. 8 Relative abundance of unbranched Leveillula taurica conidiophores formed by mycelia in leaves of Galphimia glauca of different R W C's

## F. STUDIES ON THE LEVEL OF ENDOGENOUS SUBSTRATES AND GERMINATION CAPACITY OF *L. TAURICA* CONIDIA FORMED BY MYCELIUM GROWING IN MATURE LEAVES OF DIFFERENT R.W.C.

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### i. Relative amount of endogenous substrate of the conidia.

The percentages of the conidia from leaves of different R.W.C. which germinated at 30$^0$C at 100% R.H. over 48 hours are shown in Table 16. Observations were not possible beyond 48 hours as the hyphae broke up after that. The mean maximum germ tube length of 81.7 μm produced by conidia formed on leaves of 66.7 percent R.W.C. was markedly inferior to lengths of 143.2-172.8 μm of the other categories of conidia.

Values of percentage germination, on the other hand, divided the four sets of conidia into three groups. Conidia formed at 80.6 and 66.7 percent R.W.C. constituted the upper and lower extremes of 43.9 and 10.4 percent germination, respectively, while those of leaves of 74.4 and 78.4 percent R.W.C. formed an intermediate group.

### ii. Germination of the conidia at humidities of zero to 100% R.H.

The conidia of all the four categories germinated at all humidities from zero to 100% R.H. as shown in Table 17 and Fig.9. The data showed that:

a. the conidia germinated best at 100% R.H. and percentage germination declined with decreasing percentage relative humidity.

b. the ratio of percentage germination at 100% R.H. and 0.0% R.H. differed among the different categories of conidia. It was:

20.1 to 6.0 percent for conidia of leaves of 80.6 percent R.W.C., 14.7 to 1.4 percent for conidia of leaves of 78.4 percent R.W.C.,

13.3 to 1.4 percent for conidia of leaves of 74.4 percent R.W.C., and

12.5 to 4.0 percent for conidia of leaves of 66.7 percent R.W.C.

c. Germ tubes formed in 24 hours were also longest at 100% R.H. and decreased in length with decreasing percentage relative humidity.

d. The ratios of the germ tube lengths at 100% R.H. and 0.0% R.H. were, however, fairly close:

116.6 to 38.8 μm for conidia of leaves of 80.6 percent R.W.C.,

114.9 to 34.3 μm for conidia of leaves of 78.4 percent R.W.C.,

92.6 to 24.0 μm for conidia of leaves of 74.4 percent R.W.C. and

88.6 to 36.3 μm for conidia of leaves of 66.7 percent R.W.C.

e. At the higher humidities of 85.2-100% R.H. the higher the percentage R.W.C. of the host leaf, the greater the percentage germination. But this trend was not maintained at the lower humidities of zero -62.5% R.H. and this observation would be discussed later in the thesis.

Table 16.

**Germination and growth of germ tubes of Conidia of *Leveillula taurica* formed by mycelium growing in leaves of host, *Galphimia glauca*, of different R.W.C. over 48 hours at 100% R.H. 30$^0$C**

| R.W.C of leaf (%) | Percentage germination | Mean Germ Tube Length ± S.E (μm) |
|---|---|---|
| 80.6 | 43.9 | 172.8 ± 0.39 |
| 78.4 | 20.0 | 143.2 ± 0.12 |
| 74.4 | 28.8 | 166.3 ± 0.03 |
| 66.7 | 10.4 | 81.7 ± 0.11 |

Table 17

Percentage germination of conidia of *Leveillula taurica,* formed by mycelium growing in *Galphimia glauca* leaves of different R.W.C., at different Relative Humidities at 30$^0$C in hours.

| Relative humidity (%) | R.W.C. (%) of Galphimia glauca leaves | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 80.6 | | 78.4 | | 74.4 | | 66.7 | |
| | Percentage Germination | Mean germ tube length (μm) | Percentage Germination | Mean germ tube length (μm) | Percentage Germination | Mean germ tube length (μm) | Percentage Germination | Mean germ tube length (μm) |
| 100 | 20.1 | 116.6 | 14.7 | 114.9 | 13.3 | 92.6 | 12.5 | 88.6 |
| 92.8 | 15.4 | 74.9 | 14.1 | 81.9 | 13.2 | 89.9 | 11.0 | 76.6 |
| 85.2 | 17.4 | 74.4 | 12.0 | 63.3 | 11.6 | 65.0 | 10.4 | 61.1 |
| 62.5 | 8.1 | 54.9 | 7.9 | 58.3 | 5.4 | 57.3 | 10.0 | 67.9 |
| 44.8 | 6.9 | 58.3 | 7.3 | 54.9 | 4.5 | 54.6 | 8.1 | 51.9 |
| 16.9 | 6.4 | 42.6 | 2.7 | 53.3 | 2.0 | 45.3 | 5.6 | 48.6 |
| 0.0 | 6.0 | 38.8 | 1.4 | 34.3 | 1.4 | 24.0 | 4.0 | 36.3 |

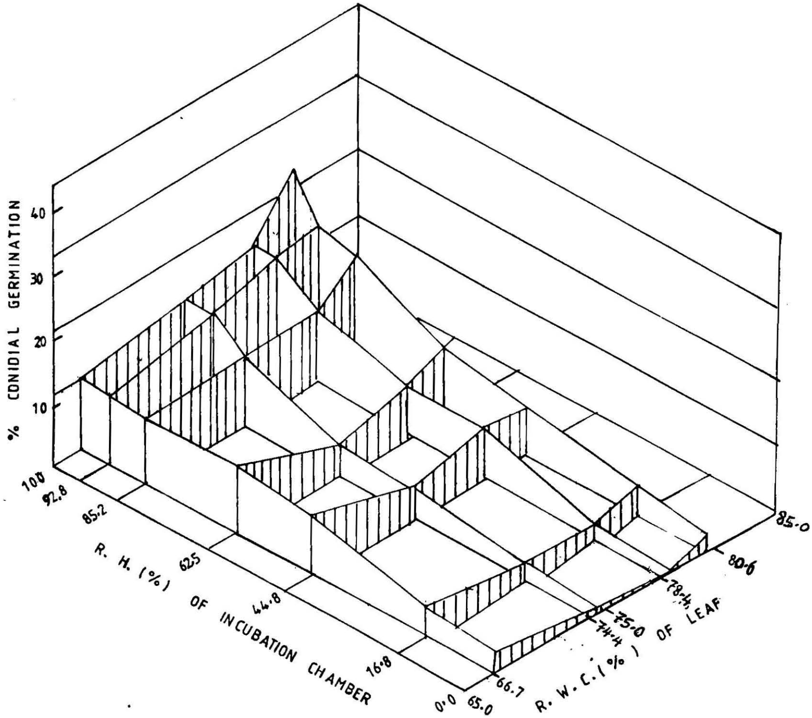


Fig. 9 Germination of Conidia of Leveillula taurica formed by mycelium growing in Galphimia glauca leaves of different R. W. C. at different Relative humidities at 30° In 24 hours

## G. STUDIES ON SIZE OF HAUSTORIA OF *L. TAURICA* MYCELIA OF DIFFERENT RELATIVE WATER CONTENT INDUCED BY ADJUSTING HOST LEAVES TO DIFFERENT R.W.C.

*L. taurica* mycelia growing in leaves of different Relative Water Content differed in their development. Fig.10 shows clearly that in leaves of greatest water stress, *L. taurica* produced smaller haustoria and under conditions of higher water content the haustoria grew larger in size. Quantitative data obtained by measuring the diameters of the haustoria are shown in Table 18. The values were significantly different from each other. An increase of the R.W.C. from 66.7 to 80.6 percent R.W.C. caused an increase of slightly more than 40 percent increase in size of the haustoria of 10.0 to 14.3 μm.

Table 18

**Size of haustoria formed by mycelium of *Leveillula taurica* growing in leaves of *Galphimia glauca* of different R.W.C.**

| R.W.C. % | Mean Diameter of 20 Haustoria ± S.E. (μm) |
|---|---|
| 80.6 | 14.3 ± 0.06d |
| 78.4 | 12.7 ± 0.16c |
| 74.4 | 11.3 ± 0.06b |
| 66.7 | 10.0 ± 0.00a |

*By calculated Confidence Limits, figures bearing the same letters are not significantly different at 95% level

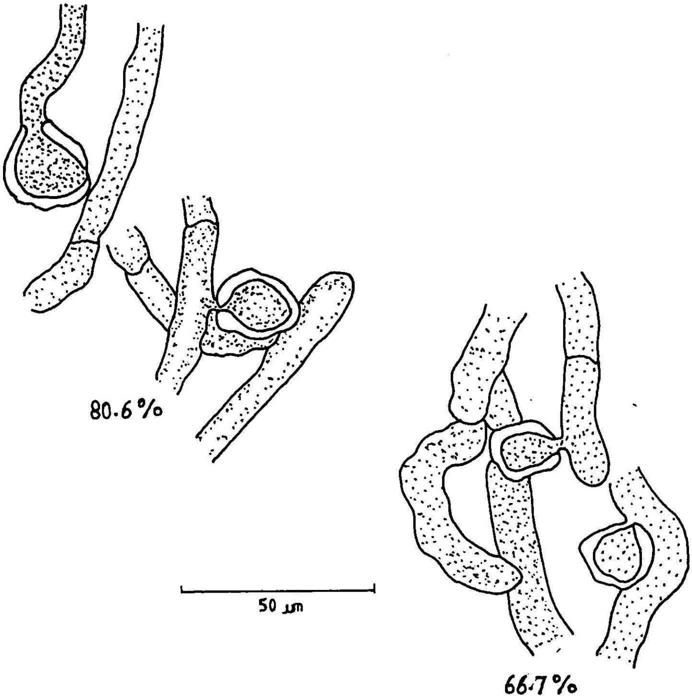


Fig.10 Camera lucida drawings of selected haustoria formed by mycelium growing in leaves of Galphimia glauca of 66.7 and 80.6 R.W.C. (%)

## H. STUDIES ON GERMINATION OF CONIDIA PRODUCED BY *L. TAURICA* MYCELIA OF DIFFERENT MOISTURE CONTENT ON UNINFECTED LEAVES OF *G. GLAUCA* OF DIFFERENT R.W.C.

The results presented in Table 19 show that although the R.W.C. of both the leaves of the source of conidia and of the inoculated plant affected conidial germination and germ tube length, the R.W.C. of the leaves of the inoculated plant seemed to exert more influence. For example, conidia formed on leaves of 80.6 percent R.W.C and inoculated on the leaves of 66.7 percent R.W.C. gave a percentage germination of 22.4 percent, while the reverse combination gave 30.0 percentage germination. It was clear that combinations involving 74.4 and 80.6 percent R.W.C. (ie. 80.6 - 80.6; 80.6 - 74.4 and 74.4 - 80.6 percent R.W.C.) gave better percentage germination and longer germ tubes than the other combinations.

The camera lucida drawing in Fig. 11 indicates that there is no evident relationship between the epidermal cells and stomata of the host leaf, and the course of growth of the germ tubes. The paths of growing germ tubes on the leaf surface were random.

Table 19

**Germination of conidia of *Leveillula taurica* from *Galphimia glauca* leaves of different R.W.C. on leaves of *Galphimia glauca* of similar or different R.W.C. at $30^0$c in 24 hours.**

| Inoculation Combination | | | |
|---|---|---|---|
| R.W.C. (%) of source Plant. | R.W.C. (%) of leaves of Inoculated plant | Percentage germination | Mean germ tube length (μm) |
| 80.6 | 80.6 | 40.0 | 81.6 |
| 80.6 | 74.4 | 34.4 | 79.5 |
| 80.6 | 66.7 | 22.4 | 65.6 |
| 74.4 | 80.6 | 42.1 | 77.9 |
| 74.4 | 74.4 | 37.5 | 62.7 |
| 66.7 | 80.6 | 30.0 | 67.7 |
| 66.7 | 66.7 | 19.0 | 53.3 |

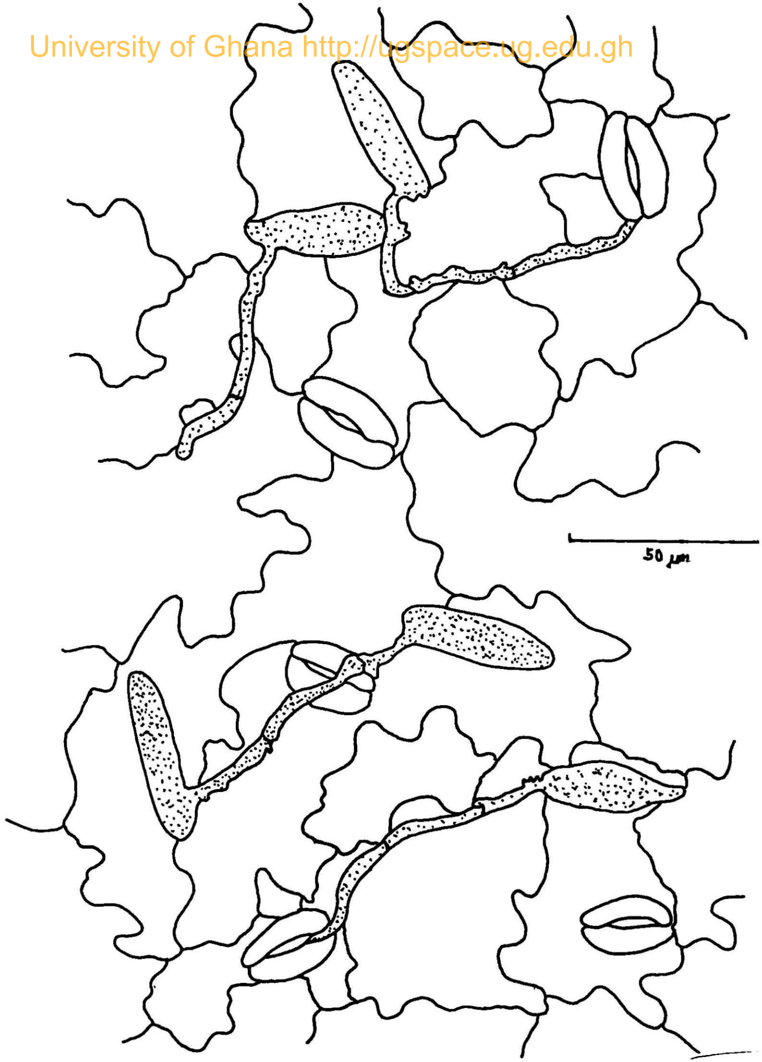


Fig.11 Camera lucida drawing of Galphimia glauca leave surface showing germinated conidia of Leveillula taurica from Galphimia glauca leaves of 80.6 R W C (%) on Galphimia glaucia leaves of same R. W. C. incubated at 30°C for 24 hours.

## I. STUDIES ON INFECTION RATE OF *G. GLAUCA* LEAVES OF DIFFERENT R.W.C. SURFACE - INOCULATED WITH *L. TAURICA* CONIDIA.

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The results of Table 20 shows that the time of infection was shortest (4 days) for conidia from leaves of 80.6 and 74.4 percent R.W.C. inoculated onto leaves of 80.6 percent R.W.C. Infection occurred in 5 days in the rest of the tests except the test involving R.W.C. of 66.7 percent of both source and inoculated plants which took as long as 7 days for infection to occur.

Table 20

Infection of *Galphimia glauca* leaves of different R.W.C., surface-inoculated with *Leveillula taurica* conidia from *Galphimia glauca* leaves of similar or different R.W.C.

| Inoculation Combination | | |
|---|---|---|
| R.W.C. (%) of leaves of source plant. | R.W.C. (%) of leaves of Inoculated plant. | Infection time (Days) |
| 80.6 | 80.6 | 4 |
| 80.6 | 74.4 | 5 |
| 80.6 | 66.7 | 5 |
| 74.4 | 80.6 | 4 |
| 74.4 | 74.4 | 5 |
| 66.7 | 80.6 | 5 |
| 66.7 | 66.7 | 7 |

## J. STUDIES ON THE EXTENT OF GROWTH OF *LEVEILLULA TAURICA* MYCELIUM IN THE HOST PLANT.

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Exhaustive study of sections of stems bearing diseased leaves was carried out. *L. taurica* could not be detected in the stem. Evidently, the mycelium does not invade the stem.

# V. GENERAL DISCUSSION

*Leveillula taurica* is a powdery mildew with a very wide host range. It has been recorded on a total of about 1000 flowering plant species, in 390 genera of 74 families which include both dicotyledonous and monocotyledonous species, and herbaceous plants, shrubs and trees (see Table 1). Some of the hosts are of economic importance.

Actual measurements of economic losses due to *Leveillula* attack have, however, been reported only for a small number of crops. Significant reductions, for instance, have been found by Bremer et al. (1947) in the yield of egg plants, in Turkey, as a consequence of as high as 90 percent defoliation. Unsightly mildew lesions and defoliation have caused considerable economic losses in products of such horticultural plants as nasturtium (*Tropaeolum majus*), hollyhock (*Althaea spp.*), calla lily (*Zantedeschia* sp.), and various species of *Euphorbia* (Palti, 1988). Palti (op. cit.) further pointed out that the numerous reports of fungicidal tests against *Leveillula taurica* indicate that the mildew causes enough loss of income to be worth the expense of control.

There is only one report on the quantitative assessment of the effect of *L. taurica* on a host in Ghana. Brown (1976) made the assessment on five local varieties of pepper, namely the Long-fruited, Large-fruited, Medium-fruited and Round-fruited varieties of *Capsicum annuum* and the small-fruited variety of *Capsicum frutescence.* He found that infection was significantly higher in the rainy season than in the dry season or harmattan. In September 1975 the percentage of leaves of the Large-fruited, Long-fruited, Medium-fruited, Round-fruited and Small-fruited varieties infected was 37.4, 61.9, 42.7, 43.8 and 19.5 percent, respectively.

There have hitherto been only five reported hosts of *L. taurica* in Ghana. Among these, *Capsicum annuum,* and *Capsicum frutescence* shed leaves prematurely on infection (Brown, 1976) while the remaining three *Lycopersicum esculentum, Solanum macrocarpon* and *Solanum melongena* do not (Clerk, G.C., personal communication). The present study of the disease in the ornamental plant, *Galphimia glauca,* which is a new found host in the country has shown that infected *G. glauca* plants shed leaves prematurely. Heavy leaf abscission and chlorosis of the leaves would adversely affect fruiting and the amount of seeds which would be available for propagation.

Infection of *G. glauca* plants was always detected first on the older leaves and as the disease progresses, whitish lesions are formed on both the abaxial and adaxial surfaces of the leaves. Spreading lesions on a leaf eventually merge and the entire leaf surface may be covered by a crop of conidiophores bearing masses of hyaline conidia (see Plate 2). It was estimated in May 1997 in the rainy season that 52.2 to 73.0 percent of the leaves could be affected (see Table 3).

Despite this heavy infection, the fungus did not attack the floral parts of *G. glauca* nor the stem. Infact, only seven species parasitized by *L taurica* among the overwhelmingly large numbers of host species have shown infection of the flowers (Cirulli, 1975, Palti, 1959, 1988; Rayss, 1940; Saad *et al*., 1972). The epidermis of petals has stomata through which the conidiophores could emerge. The colour of petals results from pigments in chromoplasts (carotenoids) and in the cell sap (anthocyanins) and from various modifying conditions such as, for example, acidity of the cell sap (Paech, 1955). The peculiar internal environment may not be suitable to *L. taurica.*

The infected leaf becomes prematurely chlorotic with isolated patches of green tissue forming what is termed 'green islands' (see Plate 3). *G. glauca* produces numerous small yellow flowers (see Plate 1). The yellowing leaves blend fairly well with the yellow blossom and do not impair unduly the attractiveness of the plant, which is extensively planted in gardens in Ghana.

This is the first observation of 'green island' in *L. taurica* - infected plants. Another powdery mildew, *Phyllactinia gutatta* (= *corylea)* also causes premature chlorosis of pawpaw (*Carica papaya*) leaves with 'green island' (Ankora, 1968), while 'green islands' accompany many rust infections. Király, Hammady and Pozsár (1967) had shown that leaves of broad bean (*Vicia faba*) and bean

(*Phaseolus vulgaris)* infected by the rust (*Uromyces*) formed 'green islands', which contained higher contents of cytokinins than healthy leaves. They suggested that the higher levels of cytokinins delayed senescence and preserved the cells of the 'green island'. 'Green island' formation may be the consequence of the creation of a site of active metabolism - a nutrient sink. 'Green islands' occur in a senescing tissue, which has virtually lost its usefulness as a photosynthesizing organ and is metabolically relatively inactive because, according to Parry (1990) the presence of a biotrophic, metabolically active pathogen (nutrient sink) may simply attract nutrients to a leaf, which under normal circumstances would die. It is evident that the formation of 'green islands' implies the supply of labile metabolic products and the survival of obligate parasites for a limited extended period.

Allen (1942) pointed out the fact that 'Green Island' formation in mildew-infected wheat leaves does not merely represent retention of chlorophyll but they are actually areas in which the chlorophyll was destroyed and subsequently reformed. Harding, Williams and McNabola (1968) studying 'green island' formation on *Brassica juncea* cotyledons infected by *Albugo candida* concluded that although there are both chlorophyll retention and chlorophyll resynthesis at the green islands, delayed chlorophyll breakdown seemed of more importance than chlorophyll resynthesis. Cytokinins applied to detached leaves or to leaves on intact plants delay leaf senescence (Fletcher, 1969). In addition, cytokinins stimulate chlorophyll production (Fletcher and McCullagh, 1971a,b; Fletcher, Teo and Ali, 1973).

Experimental evidence presently available suggests that other factors may also play a role in green island formation. Significant levels of cytokinin activity have been detected in extracts from both yellow zones surrounding infection zones and senescent healthy bean leaves (Dekhuijzen and Staples, 1968). If cytokinins play such an important role in keeping leaves or part of a leaf green one would not expect high cytokinin activity in those senescent tissues. Abscisic acid occurs abundantly in senescent leaves (Böttger, 1970) and may be one of the factors that counteract cytokinin activity in senescent healthy leaves. With regard to the resynthesis of chlorophyll, gibberellic acid may be involved because it acts synergistically with cytokinins in the synthesis of chlorophyll in Mung beans (Hole and Dodge, 1972). Green island formation then reflects disturbances in the hormone balance of the host which is triggered by obligate parasites, and as has been found, by leaf miners (*Stigmella* spp.) as well (Engelbrecht, 1971; Engelbrecht, Orban and Heese, 1969).

The survival of the strain of *L. taurica* on *G. glauca* would eventually depend on the conidia. In *G. glauca,* the mycelium is apparently restricted to the infected leaf, which is soon shed resulting in the death of the fungus. The conidia, as had been reported for spores of other strains of *L. taurica* (Ayesu-Offei, 1966; Caesar, 1976; Nour, 1958), germinated at all humidities and would not store under field conditions. Knowledge on the germination capacity and the effects of both environmental and endogenous factors, which have been studied in this work, will contribute to understanding conditions, which favour the spread of the disease.

Germination of conidia, formed on *G. glauca* plants watered daily, in light at humidities of 0.0, 16.9, 44.8, 62.5, 85.2, 92.8 and 100% R.H. was 6.0, 6.4, 6.9, 8.1, 17.4, 15.4, and 20.1 percent respectively (see Table 17). Ayesu-Offei (1966) recorded an even lesser percentage germination at the lower humidities. He reported values of 3.1, 2.7, 5.6, and 5.7 percent germination, respectively, at 0.0, 20, 40, and 60% R.H. That was one difference found between the conidia of *G. glauca* strain and the pepper strain. In addition the conidia differed in size; the conical conidia of the isolate of pepper were longer (mean of 65.7μm) but the cylindrical conidia were shorter (mean of 58.6μm) (Ayesu-offei, 1966) compared to the conidia of *G. glauca* plants (see Table 5), with mean lengths of 55.8μm and 63.8μm for the conical and cylindrical conidia, respectively. Since it had been found that conidial germination was better in light than in dark (see Table 7), the conidia would germinate even poorer in dark at the lower humidities from zero to 62.5% R.H.

Another difference between the *L. taurica* strains from *G. glauca* and pepper was that the germ tubes of the *G. glauca* strain grew longer in light (see Table 7) while they were shorter in isolate studied by Ayesu-Offei (1966). This difference cannot be readily explained and this aspect of the behaviour of the conidia should be further investigated using strains of *L. taurica* from the other host species as well. Length of the germ tubes is important during infection. The germ tubes which enter the host through the stoma (Ayesu-Offei, 1966) are not directed by any stimulus during growth on the leaf surface as shown by the camera Lucida drawings in Fig. 11. The longer the germ tube, the greater the chance of encountering a stoma. The role of light in infection would, therefore, be three-fold. First, conidia formed in the light and arriving on a fresh leaf surface in the night would be least damaging and they would germinate poorest as indicated by the results in Table 9. Secondly, conidia, irrespective of the time of formation, arriving at the leaf surface during the day would be more infective than the same number of conidia arriving during the night. Thirdly, leaves fully exposed to the sun and, consequently, to higher light intensity would be more heavily infected than leaves shaded by other leaves and receiving diminished light intensity. For, the data in Table 8 show that germination in light of 22 lux was only 21.2 percent compared to 36.9 percent germination in light of 76 lux.

The stimulating effect of light in spore germination remains to be explained, not only in *L. taurica* but in other fungus species such as *Alternaria oleracea* (Neergaard, 1941). Cherewick (1944) had shown that temperature affects the mechanism involved. He reported that light depressed germination of *Erysiphe graminis* conidia at 30 and 35$^0$C whereas it enhanced germination at 10 - 25$^0$C. For any spore, there would be a threshold of light intensity above which inhibition of germination would set in due to the accompanying heating effect of the high light intensity.

The amount of water available in the *G. glauca* plants had a profound effect on the conidia. This could be inferred most clearly from the amount of water accumulated in the mature spores.

Plants of different Relative Water Content were kept together, and therefore, in an atmosphere of the same relative humidity. When conidia formed by plants of 66.7, 74.4, 78.4, and 80.6 R.W.C. (%) were stored at 0.0% R.H., all the conidia from 66.7 R.W.C (%) plants were shrivelled after 50 minutes, while only 20.0 and 7.6 percent of the conidia from the 78.4 and 80.6 R.W.C. (%) plants, respectively had shrivelled (see Table 13). It means that many of the conidia formed on plants of low R.W.C. (%), as would occur during the harmattan season, would perish while being dispersed through the dry atmosphere of that season. This may be one of the reasons for the observed lower infection rate, during this investigation in the dry season than in the rainy season. An interesting and pertinent observation was made by Clerk and Ankora (1971). They found that, even though substantial numbers of conidia of the powdery mildew, *Phyllactinia corylea*, germinated at all relative humidities from zero to 100% R.H., those germinating at the lower humidities died soon after germination. Germination at 0.0 - 100% R.H ranged from 50.2 to 56.9 percent in six hours and within the next six hours all the germinated conidia at 0.0% R.H. died, followed by death of all the germinated conidia at 12 - 53.4% R.H. a few hours later.

Reduced water content of the host leaves would trigger off a series of events, which would fortunately reduce infection. The reduced water content of the leaves would affect metabolism of the plant. The fungus would receive less amount of water and reduced quantities of nutrients. It is possible that the quantity of nutrient entering the parasite might be altered as well. Water stress would affect metabolism of the plant and the liable metabolic products, which sustain an obligate parasite. It is known that plants respond to most environmental stresses by changing their hormonal balance, frequently producing more abscisic acid and often less cytokinin (Chapin, Walter and Clarkson, 1988). This alteration of hormonal balance is a direct factor that causes reduction of growth in water stressed plants (Davis, et all.1986). The changes in hormonal balance cause changes in cell wall extensibility and, therefore, growth (Blackman and Davis, 1984).

During water stress, abscissic acid is synthesized in increased amounts in roots as a hormonal signal which is transported through the transpirational stream in the xylem and effectively closes the stomata quite independently of water status of the leaf (Zhang and Davis, 1987).

Proline and certain compounds accumulate in plants under stress. Proline and betaine concentrations increased significantly in barley seedlings subjected to water stress (Zuniga, et all. 1990). The accumulation of amino acids, particularly proline, is regarded as providing energy and nitrogen after the stress has ended, stabilizing membrane and acting as a neutral osmoticum (Barnett and Naylor, 1966). Proline is readily synthesized and accumulated from glutamic acid.

The consequence of decreased leaf water content in this investigation was manifested in many different ways. Growth of the fungus was depressed. For example, the size of the haustorium significantly decreased with decreasing leaf Relative Water Content (see Table 18 and Fig. 10), conidiophore branching became more profuse as the Relative Water Content increased (see Table 15) and longer conidia were formed at higher leaf Relative Water Content (see Tables 11 and 14, and Fig. 5).

The vigour of the conidia (germination capacity) and the amount of nutrients in the conidia were also affected. Germination, in 48 hours, of conidia formed on leaves of 80.6% Relative Water Content was four-fold (43.9 and 48.9 percent) of that of conidia formed on leaves of 66.7% Relative Water Content (10.4 and 12.2 percent) (see Tables 12 and 16), while the endogenous nutrients in the corresponding conidia were able to support development of maximum germ tube lengths of 172.8 - 188.5μm and 72.6 - 81.7μm, respectively (see Tables 12 and 16). Growth of hyphae does not depend on the amount of available substrate only, but also on the efficiency of the various metabolic processes involved, which might have been determined by the conditions under which the conidia were formed. The data in Table 12 and 16 were obtained from germination tests using conidia from mycelia subjected to the same levels of water stress but at different times in relation to the time of sporulation. The effect of the treatment might have been more intense in the second experiment in which the mycelium grew in water-stressed leaves (showing percentage germination of conidia of 10.4, 28.8, 20.0 and 43.9 for conidia of leaves of 66.7, 74.4, 78.4, and 80.6% Relative Water Content, respectively) (see Table 16), compared with the effect in the first experiment in which water stress was imposed immediately prior to sporulation (showing corresponding percentage germination of 12.2, 34.6, 31.5 and 48.9 for the four levels of Relative Water Content) (see Table 12).

It is reasonable to expect that conidia with diminished capabilities would take longer time to cause disease, and particularly so, if conditions in the new host were sub-optimal. It explains why it took 7 days for infection to occur in plants of 66.7% Relative Water Content inoculated with conidia formed on plants of 66.7% Relative Water Content, while it took only 4 days in plants of 80.6% Relative Water Content inoculated with conidia formed on plants of 80.6% Relative Water Content (see Table 20). Conidia formed in the harmattan season would be weaker and the plants, in turn, would present only partially open stomata because of the accumulation of abscisic acic (Zhang and Davies, 1987) and would provide less congenial internal environment for infection due to the accumulation of proline and other compounds.

Water stress retards plant growth (Kozlowski, 1968; Kramer, 1969). This factor was avoided in this study by using leaves, which had developed fully. Apart from the changes which had earlier been mentioned that would occur in water stressed leaves, other important consequences of water stress would be the reduction in photosynthesis, decrease in translocation of carbohydrates and growth regulators and the decline in respiration rate. According to Boyer (1970), for example, the rate of respiration of corn, soyabean and sunflower plants at -16 bars was exactly half the rate at -4 bars. Neither the photosynthetic nor respiration rate of the *G. glauca* leaves was measured in the present studies. The extent of reduction of these processes in leaves of 78.4, 74.4 and 66.7% Relative Water Content was, therefore, not known. However, a reduction in photosynthetic rate significant enough to affect the amount of nutrients reaching the fungus should be expected.

Ayres (1977) found that water stress imposed on pea plants caused decreased mycelial growth and spore production in *Erysiphe pisi* parasitizing the plant as leaf water potential decreased from -4.5 to -9.5 bars. *E. pisi* is ectotrophic and the hyphae on the leaf surface could be observed under the microscope and the increase in length of the growing hyphal tip could be measured. There was no relatively simple means by which mycelial growth of *L. taurica* could be studied because its mycelium is endotrophic. The rate of sporulation of individual conidiophores was also not attempted even though this had been done in this laboratory by continuous observation of conidiophores under the microscope over 24 hours by Ankora (1968) and Teyegaga (1969) studying *Phyllactinia corylea* and *L. taurica*, respectively. The intact mildewed leaves were folded and held onto the microscope stage with cellotape, bringing the projecting conidiophores along the folded edge in line with the objective. *G. glauca* plants growing in pots and used in the present investigation were too short to get the leaves of the plants fixed onto the microscope stage for direct observation. Periodic harvesting of mature conidia by sucking with a vacuum pump from the disease lesion used in some studies (eg. Woolacott and Ayres, 1984) would not provide information on productivity of the individual conidiophore. Sufficient evidence has, however, been obtained to support the view that *L. taurica* was adversely affected by water stress imposed on the host plant and the degree of the effect assessed quantitatively by other parameters such as, size of the conidia, germination capacity of the conidia, branching of the conidiophores and size of the haustoria.

Nour (1958) had demostrated the ability of conidia of *L. taurica* from one host species to infect other hosts belonging to different families. Several cultivars of Egyptian cotton (*Gossypium barbadense)* and of American cotton (*Gossypium hirsutum*) became infected when artificially inoculated from diseased leaves of *Euphorbia heterophylla* or *Faba bona.* Cross-inoculations in the reverse direction were equally successful. It remains to be seen in future studies whether crossinfection is possible among the various hosts of *L. taurica* in Ghana.

Furthermore, since it has been shown that water stress imposed on the host plant could affect the growth of the fungus and reduce the vigour, and thereby the infectivity, of the conidia, watering regimes that would impose moderate water stress without restraining plant development unduly could be adopted to weaken the parasite and contain infection.

# IV. SUMMARY

1. Powdery mildew disease of *Galphimia glauca* Cav. is caused by *Leveillula taurica*.
2. Lesions of *L. taurica* conidiophores and conidia occurred on both the abaxial and adaxial surfaces of host leaves at advanced stage of the disease.
3. Infected leaves become prematurely chlorotic with scattered 'green islands'.
4. Infection of host plants was very heavy (63% of leaves infected) in the rainy season and very scanty in the dry season.
5. *L. taurica* formed globose haustoria, measuring from 6.7 to 23.3μm in diameter with a mean of 14.2μm, in the host mesophyll cells.
6. *L. taurica* formed both conical and cylindrical conidia on *G. glauca* leaves. The cylindrical conidia had a mean length of 63.8μm and mean width of 12.7μm. The corresponding values for the conical conidia were 55.8μm and 11.6μm, respectively.
7. Both unbranched and branched conidiophores were formed. The lengths of unbranched or simple conidiophores ranged from 40.0 to 189.8μm, with a mean length of 101.4μm.
8. The maximum number of branches observed on a conidiophore was four.
9. The conidia germinated better in light of 76 lux than in dark, particularly after 12, 24 and 36 hours incubation.
10. Germination of conical and cylindrical conidia was practically the same.
11. Longer germ tubes were formed by conidia in light. The mean germ tube lengths of conical and cylindrical conidia incubated in light were 102.2 and 100.9μm and those in dark were 76.9 and 65.1μm, respectively.
12. The percentage germination of conidia formed in light of 76 lux at 100% R.H. for 24 hours

was 20.8 percent and the mean length of the germ tubes was 69.1μm, while the percentage germination of conidia formed in dark and germinated in dark was 11.4 percent and the mean length of the germ tubes was 43.4μm.

13. Different Relative Water Contents were established in *G. glauca* leaves by watering the plants at different frequencies as follows:

| | | |
|---|---|---|
| Daily watering | : | 80.6% R.W.C |
| Watered once in 2 days | : | 78.4% R.W.C |
| Watered once in 3 days | : | 74.4% R.W.C |
| Watered once in 4 days | : | 66.7% R.W.C |

14. Mean size of the conidia decreased with increasing water stress:

    a. At 80.6% R.W.C.: conical conidia were 54.8μm and cylindrical conidia were 55.6μm long

    b. At 78.4% R.W.C.: conical conidia were 52.4μm and cylindrical conidia were 55.7μm long.

    c. At 74.4% R.W.C.: conical conidia were 51.4μm and cylindrical conidia were 50.6μm long.

    d. At 66.7% R.W.C.: conical conidia were 49.4μm and cylindrical conidia were 51.5μm long.

15. In an atmosphere of 100% R.H., percentage germination of the conidia decreased with decreasing percentage R.W.C. of the host leaves on which they were formed. The percentage germination after 48 hours were as follows:

    a. 80.6% R.W.C.: 48.9 percent.

    b. 78.4% R.W.C.: 31.5 percent.

    c. 74.4% R.W.C.: 34.6 percent.

d. 66.7% R.W.C.: 12.2 percent.

16. The mean lengths of germ tubes produced after 48 hours by the conidia formed by leaves of different % R.W.C. were:

a. 80.6% R.W.C.: 188.5μm

b. 78.4% R.W.C.: 179.4μm

c. 74.4% R.W.C.: 94.5μm

d. 66.7% R.W.C.: 72.6μm

17. Conidia formed on leaves of different % R.W.C. shrivelled at different rates in an atmosphere of 0.0% R.H. The percentage of conidia shrivelled after storage for 10, 60, 120 and 180 minutes was as follows:

a. Conidia of 80.6% R.W.C.: 2.9, 9.5, 42.6 and 87.0%, respectively.

b. Conidia of 78.4% R.W.C.: 6.3, 36.8, 77.1 and 90.0%, respectively.

c. Conidia of 74.4% R.W.C.: 20.8, 65.3, 89.5 and 97.1%, respectively.

d. Conidia of 66.7% R.W.C.: 71.4, 100, 100, and 100%, respectively.

18. R.W.C. of the host leaves affected conidiophore length and branching. The higher the % R.W.C., the longer the conidiophores and greater the number of branches.

19. Conidia formed on leaves of the different % R.W.C germinated at all humidities from zero to 100% R.H. and percentage germination declined with decreasing percentage relative humidity. Germ tubes formed in 24 hours were also longest at 100% R.H. and decreased in length with decreasing percentage humidity.

20. Growth of *L. taurica* in leaves of different Relative Water Content showed some differences. Diameter of the haustoria decreased with increasing water stress.

a. Mean haustorial diameter in leaves of 66.7% R.W.C. was 10.0μm.

b. Mean haustorial diameter in leaves of 74.4% R.W.C. was 11.3μm.

c. Mean haustorial diameter in leaves of 78.4% R.W.C. was 12.7μm.

d. Mean haustorial diameter in leaves of 80.6% R.W.C. was 14.3μm.

21. The R.W.C.'s of the infected leaves and of the plants inoculated with the conidia influence germination of the conidia. Using leaves of 66.7, 74.4 and 80.6% R.W.C., combination between those of 74.4 and 80.6% R.W.C. encouraged higher germination than any other combination.

22. The germ tubes grew in all directions on the host leaf surface and the direction of movement was not in response to any stimulus or topography of the leaf surface.

23. Infection occurred fastest on leaves of 80.6 percent R.W.C. inoculated with conidia from leaves of either 80.6 and 78.4 percent R.W.C. and slowest on leaves of 66.7% R.W.C. inoculated with conidia from leaves of 66.7% R.W.C.

24. Lesions were absent on the flowers.

25. Sections of the stem did not reveal the presence of any mycelium.

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APPENDIX A.

Relative abundance (Percentage Frequency) of unbranched *Leveillula taurica* conidiophores in different class-lengths formed by mycelium on *Galphimia glauca* leaves.

| Class-Lengths (μm) | | | Percentage Frequency |
|---|---|---|---|
| 20.1 | - | 50.0 | 10 |
| 50.1 | - | 80.8 | 30 |
| 80.1 | - | 110.0 | 30 |
| 110.1 | - | 140.0 | 10 |
| 140.1 | - | 170.0 | 10 |
| 170.1 | - | 200.0 | 10 |
| Mean ± S.E | | 101.4 ± 0.02 | |

APPENDIX B.

Relative abundance (Percentage Frequency) of lengths of *unbranched Leveillula taurica* conidiophores formed by mycelia in leaves of *Galphimia glauca* of different R.W.C's.

| Class-Lengths (μm) | Calculated R.W.C. of leaves. | | | |
|---|---|---|---|---|
| | 80.6 | 78.4 | 74.4 | 66.7 |
| 20.1 - 50.0 | 0 | 0 | 5 | 0 |
| 50.1 - 80.0 | 0 | 0 | 5 | 5 |
| 80.1 - 110.0 | 0 | 20 | 25 | 10 |
| 110.1 - 140.0 | 20 | 15 | 40 | 35 |
| 140.1 - 170.0 | 20 | 50 | 20 | 25 |
| 170.1 - 200.0 | 60 | 15 | 5 | 25 |
| Mean Length ± S.E. | 180.4±1.50 | 142.3±1.20 | 123.4±2.20 | 101.7±0.15 |