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COMPARATIVE STUDIES ON SOME BIOLOGY AND PATHOLOGY OF CORN AND BROOM CORN ISOLATES OF *EXSEROHILUM TURCICUM* (PASS) LEONARD & SUGGS[1]

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Abstract

Exserohilum turcicum (Pass) Leonard & Suggs, the causal fungus of northern corn leaf blight and leaf blight of sorghum, however, is only known to occur on corn and broom corn in Taiwan. Corn isolates and broom corn isolates of ***E. turcicum*** were proved to be independent races, i. e., corn isolates could not infect broom corn and broom corn isolates could not infect corn. In general, morphology of these two races is similar in shape and size of conidia. However, the length of conidiophores is significantly different; conidiophores of corn isolate are longer than those of broom corn isolates. Both races were cross-fertile and formed pseudothecia of ***Setosphaeria turcica*** (Luttrell) Leonard & Suggs on corn leaf section on Sach's medium 3 to 4 weeks after mating. Corn isolates of this fungus degenerated rapidly after growing on natural media such a V-8 juice agar, PDA, and malt extract agar. Conidia derived from degenerated cultures did germinated but failed going on to form a colony. The best and easy way to maintain this fungus is on its own host tissues. The lesion leaves were first air-dried, placed in a paper envelop and then kept at −20°C. Corn isolates preserved on 1% water agar showed promising to maintain its original vigor in growth and sporulation. Conidial development of this fungus was inhibited by light and efficient radiation regions ranging from blue to green. Conidiophores became irreversibly inhibited by light after about 7 days continuous irradiation.

Key words: *Exerohilum turcicum*; the anamorph; *Setosphaeria turcica*; the teleomorph; Corn; Broom Corn; Cultures preservation.

Introduction

Northern leaf blight of corn and sorghum is one of the important foliage diseases on corn and sorghum wherever corn and sorghum are cultivated. The biology

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and pathology of its causal fungus, *Exserohilum turcicum* (Pass) Leonard & Suggs, the anamorph (*Setosphaeria turcica*, the teleomorph), has been studied fairly extensively abroad, however, few studies on its biology and pathology have been conducted here in Taiwan. This fungus had been name *Helminthosporium turcicum* Pass and the perfect state (the teleomorph) was first obtained and named as *Trichometasphaeria turcica* by Luttrell in 1958. After a thorough study on conidia of those species of *Helminthosporium* with a conspicuous protrusion on basal end Leonard and Suggs (1974) regrouped them in a new genus *Exserohilum*, the anamorph, and *Setosphaeria*, the telomorph. Thus the causal fungus of northern leaf blight of corn and sorghum is *E. turcicum* (Pass) Leonard & Suggs, the anamorph and *S. turcica* (Luttrell) Leonard & Suggs, the teleomorph. Herein we report the results of our investigation on Taiwan isolates of *E. turcicum* which were isolated from various locations of this island. Contents in this paper include comparative morphology of anamorph of corn and broom corn and broom corn isolates, growth features on laboratory media, formation of telemorph and behaviour of germling hyphae on leaf surface and membrane.

Materials and Methods

Fungus Isolation

All isolation cultures were derived from single spore isolation by means of Wisconsin Keitt Method. Leaf sections with typical lesions were incubated inside a petri plate (9 cm) made moist chamber for 24 to 48 h at 25°C under dark condition. Great number of conidia produced on and around lesions. Conidia were sticked up by a fine glass needle and transferred onto 2% water agar plate surface and were rolled on agar surface to get rid of contaminated bacteria with a fine glass needle. Whole operation was undertaken under a stereomicroscope. The cleaned conidia were arranged and cut up with a flamed syringe needle and transferred to 20% V-8 juice agar plate individually. Cultures obtained were stocked in sterilized tape water or on 10% V-8 juice agar slants and placed at 15°C under dark condition.

Inoculation Tests

Inoculum was prepared by growing fungus on autoclaved corn leaf section (2 cm×) which was laid on Sach's medium in a 6 cm petri dish. Abundant conidia produced on the surface of leaf section one week after incubation at 25°C under darkness. Conidial suspensions were made by washing conidia off and made up to an appropriate concentration before used. Corn and broom corn used for inoculation to test pathogenicity of fungus isolates were grown in pots for one month in greenhouse. After inoculation by a sprayer or brush onto the leaves the inoculated

plants were incubated in moist condition to keep leaf surface wet for 48 h and then transferred to greenhouse bench for observation. No other environmental control was imposed unless otherwise stated. Temperatures were ranging from 20 to 30°C

Radiation Effect on Sporulation

General effect of radiation on conidiation was conducted by placing cultures which were active growing, either on V-8 juice agar or on autoclaved corn leaf section under continuous exposure to a cool white fluorescent lamp or placing under dark conditions by placing cultures insider a light proof film changing bag. Both corn and broom corn isolates were tested in this experiment. One week after incubation the results of the effect of radiation were examined under a dissecting stereomicroscope. The qualitative effect of radiation was carried out by growing cultures under light passing through different Corning glass filters. The results were observed one week after incubation at 25°C. Corning glass filters used were blue (Corning glass number #5543), blue (#5850), green (#5850), green (#4010), yellow (#3486), red (#2403), and red (#2408).

Pseudothecium Formation

No sexual state (the teleomorph) of *E. turcicum* has been reported here in Taiwan. It is of our interest and of importance to understand the biology particularly the inheritance of pathogenicity and potential of conidiation of this fungus. An experiment was carried out to induce pseudothecium development under laboratory conditions. This fungus is known to be a heterothallic and two single isolation cultures were dual inoculated on two ends of a corn leaf section (2.5× 2.0 cm) which was laid on Sach's medium in a 6 cm petri dish. The dual cultures were incubated in an incubator at 23°C to 25°C with 14-h photoperiod. Pseudothecium formation was examined 10 days after incubation and maturation of ascocarps was examined 3 weeks after dual cultured on corn leaf section under a microscope.

Germling Hyphal Behaviour on Corn and Broom Corn Leaf Surfaces and Cellophane Membrane

An appropriate concentrations of conidial suspensions were brushed on leaf surfaces and dusted on cellophane memberane which was autoclaved first and laid on 2% water agar plate in a 6 cm petri dish. Inoculated leaves were kept in moist condition for 24 h before moving to greenhouse bench. Conidial germination, germling hypha growth on leaf surface and penetration through cuticle and epidermal cells were examined 24 h and 48 h after inoculation. Inoculated leave were first cut into 1.5 cm long segments, discolorated and stained followed the procedures described by Brown and Shipton (1962). Cellophane membrane dusted with conidia

were examined directly under a microscope 8, 12, 16, 20 and 24 h after conidia were dusted on membrane.

Results and Discussion

Field Observations

Northern leaf blight of corn is one of concerned foliage disease in Taiwan and has been included in their breeding program in Corn Research Center, Tainan DAIS ever since the Institute established more than two decades ago. Writer in 1973 and 1976 respectively observed severe attack of *E. turcicum* on broom corn at Chiotsuong (Taipei) and Shutsu (Taichung). More than 30% of leaf surface of the leaves were covered with typical fusiform bright red necrotic lesions. Less severe one was also observed on various varieties of sweet corn planted in the experimental plots of Corn Research Center at Putsu in the early spring of 1983. No northern leaf blight has been reported on sorghum and not been found during the past 3 years of our investigation. 1984 had not been the northern leaf blight year on corn in the west coast but once was found on a variety of broom corn at Shutsu in the end of May 1984. During late April and May of 1984 two corn blight collection trips were made in the east coast of the island. Quite a few northern leaf blight on corn were obtained.

Isolation Culture Features of E. turcicum on Laboratory Media

In general most of conidia produced on corn leaf lesions germinated well and germinating hyphae grew fairly rapidly on ordinary media such as V-8 juice agar, potato dextrose agar, malt extract and corn meal agar, and developed into normal colony. However, during subsequent subcultures most of the cultures of corn isolations of *E. turcicum* frequently failed to grow and develope into normal characteristics of cultural features, i.e., growing uniformly, smoothly and rapidly and covered the whole 9 cm petri plate within one week. Instead, mycelia of subcultures stopped growing at various stage of development. Mycelia of these degenerate cultures showed a very peculiar character; usually grew into air and became spiral or curl up. Conidia produced by these degenerated cultures germinated but did not develop into a colony. Degeneration was widely occurred among the isolates obtained from all corn isolates examined. Hooker (1973) pointed out that this fungus lost pathogenicity and ability to sporulate when cultured for long period on artificial media. Our experiences showed that this fungus degenerated sooner or later after growing on ordinary laboratory media. Different isolates showed different rate of degeneration. Some of the isolates originated from the materials collected at Shintsueng (Hualien) performed fairly stable on V-8 juice agar, however, some clones

of these stable isolates also failed to maintain original vigor after few generations on laboratory media. One way we have employed and has showed promising to maintain its original vigor is that whenever subcultures were to be made they were first transferred onto 1% water agar plate, the fungus grew normally and formed a thin and smooth colony. Cultures derived from the cultures previouly grown on 1% water agar plate always grew normally on laboratory media (Fig. 1) and sporulated well after transferred and grew on corn leaf section. By way of growing cultures on 1% water agar the original cultural vigor has been maintaining fairly good as showed in Fig. 1.

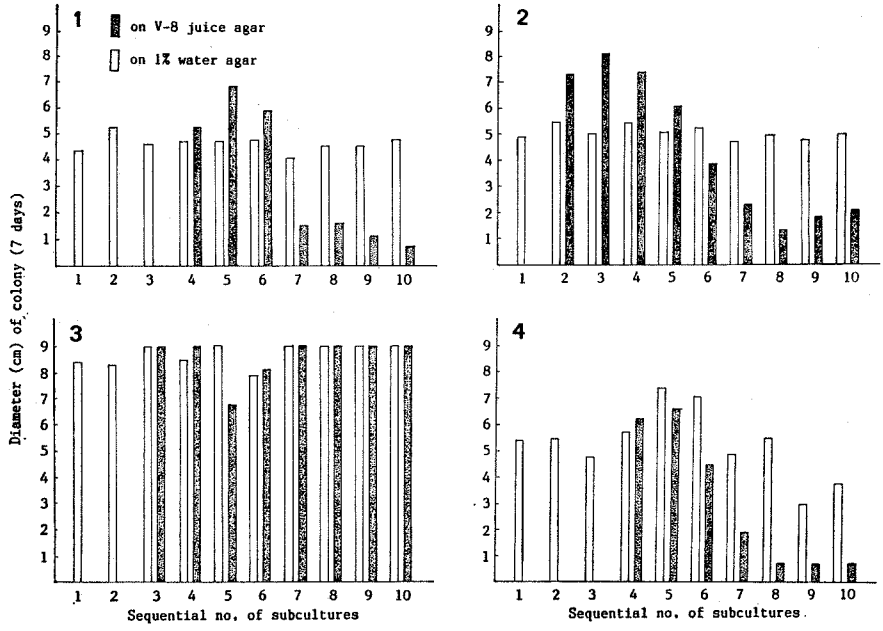

Fig. 1. Stability of growth vigor of corn isolates of ***Exserohilum turcicum*** on V-8 juice agar and 1% water agar, 1, 2, 3, and 4 are isolates CA-10, CA-20, ST-1 and ST-2 respectively.

Broom corn isolates of *E. turcicum* obtained only from Shutsu at Taichung County. The sample was collected in 1976 and 1984 respectively. Broom corn isolation cultures, in contrast to corn isolate, have maintained their original vigor after generations of culture on some laboratory media, although in a few occasions som cultures also showed degenerated.

Comparatively Morphological Investigations on Corn and Broom Corn Isolates of E. turcicum

No differences in morphology on asexual fruiting structures of this fungus

derived from different hosts have been pointed out (Tarr, 1962). The morphology and size of conidia are similar. However, we found that the length of conidiophores of corn isolates is significantly longer than those of broom corn isolates regardless of the substrates on which they developed, such as V-8 juice agar and corn leaf section (Table 1). This is the only different in morphology on corn and broom isolates of *E. turcicum*. Environmental conditions such as radiation also affect the length of condidiophores. The length of conidiophores produced under 14 h photoperiod was always longer than than those produced at darkness and those produced on corn leaf section was also longer than those produced on V-8 juice agar (Table 1)

Table 1. *The length(μm) of conidiophores of corn and broom isolates of* ***Exserohilum turcicum***

E. turcicum grew on V-8 juice agar and autoclaved corn leaf section for 5 days under continuous darkness and 14 hours photoperiod.

	Continuous darkness		14 h photoperiod	
	Broom corn	Corn	Broom corn	Corn
On V-8 juice agar	93.3±1.9	113.0±2.0*	123.0±2.3	159.2±2.5**
On corn leaf section	105.7±2.0	151.3±2.1*	164.5±2.2	195.9±2.5**

* and **: The difference in length of conidiophores of the two isolates is significant at 5% and 1% level, respectively.

Pathogenicity Tests

Varieties TAINAN 5 and TAINAN 11 of corn developed typical northern leaf blight, fusiform necrotic lesions, on leaves 2 to 3 weeks after inoculation with conidium suspensions of corn isolates. No symptoms developed except tiny flecks on corn leaves were observed when inoculated with broom corn isolates. Similarly, same type of lesions developed on broom corn leaves 2 to 3 weeks after inoculation with conidium suspension of broom corn isolates but not with corn isolates. This result suggests that corn isolates and broom corn isolates are the different races of *E. turcicum*, but morphologically they are very close.

Radiation Effect on Sporulation

Conidiation proceeded normally in dark conidtion and at 14 h photoperiod at 25°C. Continuous exposure exposure to radiation totally inhibited conidium formation but only conidiophores developed. Only light transmitted through blue and green filters inhibited conidium formation. Conidiophores which were inhibited to produce conidia by continuous illumination resumed the initiation of conidium formation

after moving the cultures to darkness. Conidiophores usually lost the potential for further differentiation and formation of conidia after relatively long period, such as longer than one week, of exposure to continuous radiation, instead the conidiophore tipe became swelling and regenerated another stage of vegetative hyphal phase.

Behaviour of Conidial Germination and Germling Hyphal Growth on Host Plant Surface and Cellophane Membrane

On host plant leaf surface: Conidia germinated bipolarly or monoplarly, in the latter case, either emerged from tip or basal end. Following germination the tips of germ tubes (or germling hypae) of broom corn isolates on broom corn leaf surface became enlarged and developed into appressoria before long. On the contrary, the growth of germling hyphae of broom corn isolate on corn leaf surface showed randomly running on leaf surface but rarely formed appressoria (Fig. 2, A, B). Conidia of corn isolates germinated well on both corn and broom corn leaf surfaces and appressoria formed and adhered on leaf surface within 24 h after conidia seeded. Frequently germ tubes developed into germling hyphae and grew further to a fairly distance from germinating conidium before its tip differentiated into an appressorium. Therefore germling hyphae of corn isolates usually were longer than those of broom corn. *On Cellophane Membrane*: Both conidia of corn and broom corn isolates germinated well 5 h after seeding on membrance which was laid on 2% water agar plate in a 6 cm Petri plate. Germling hyphal tips began to enlarge and differentiated into appressorium-like structure under either light or dark con-

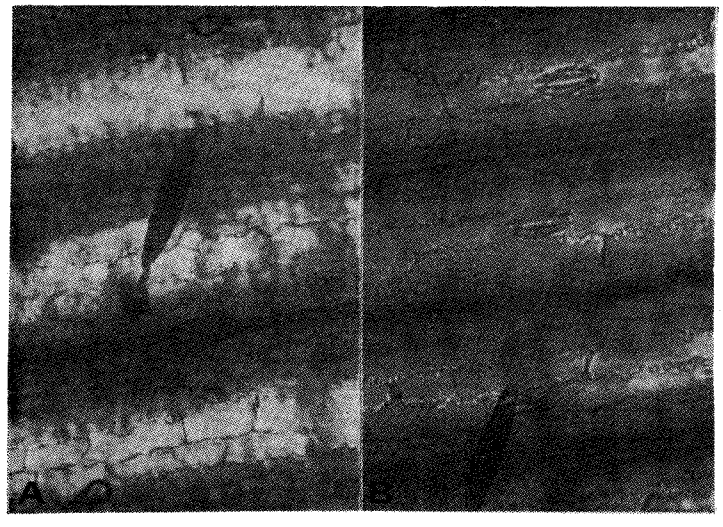

Fig. 2. Conidium germination, germling hyphal growth and appressorium formation of broom corn isolate of ***Exserohilum turcicum*** on broom corn (A) and corn (B) leaf surface. (×180)

ditions 8 h after seeding conidia on membrane surface at 25°C. Twenty four hours after seeding many appressoria produced infection hypha-like structure which penetrated the membrane and grew in the space between membrane and agar surface. The infection hypha-like structure, in most cases, was multiple finger-like (Fig. 3). The sites of the initiation of infection structure always initiated at the under side of appressoria or at one side of short branching (or knob) of germling hyphae. Besides, initiating infection structure appressoria also frequently regenerated a new vegetative hyphae and continuedly grew on membrane surface.

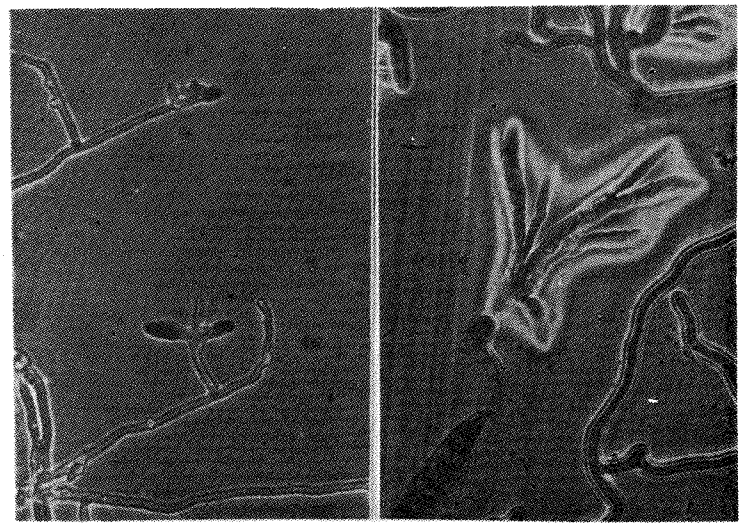

Fig. 3. Various stages of fungus development of broom corn isolate of *Exserohilum turcicum* on cellophane membrane. (×180)

Pseudothecium Formation

In preliminary test we obtained no pseudothecium formation when matings were made between isolates among broom corn cultures and the isolates obtained from sweet corn collected at Putsu. We first observed the pseudothecium formation from a mating between corn isolates collected at Chian (Hualien County) and broom corn isolates. Later pseudothecium formation was also obtained by mating broom corn isolates with some of the corn isolates collected at Shintsueng, Yuli, Sofeng besides Chian. Pseudothecia formed only on leaf section laid on Sach's medium incubated at 23 to 25°C under 14 h photoperiod but no pseudothecia formed on agar medium even adjacent to the leaf section. Pseudothecia, in most cases, formed along the line between the contact of two test cultures inoculated at both ends of corn leaf section. Fifty single ascospore isolation cultures made from a cross between a corn isolate (Chi-an) and a broom corn isolate were crossed with a broom corn isolate. Twenty pair-cultures resulted pseudothecium formation. It

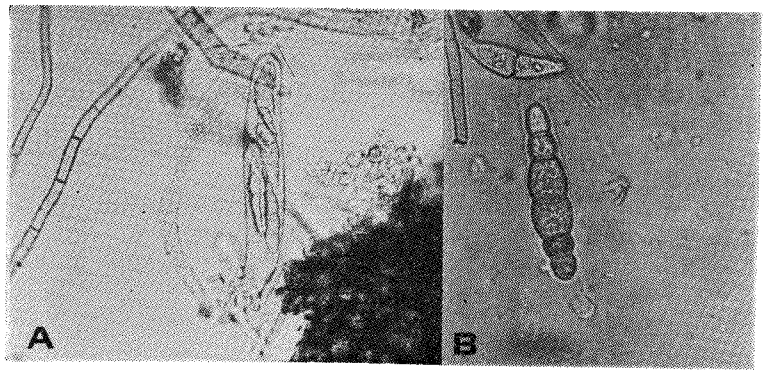

Fig. 4. Ascus amd ascospores (A) (×100) and ascospores with 5 septa (B) (×260) of *Septosphaeria turcica.*

has been well aware that mating of this group of fungi is controlled by a pair of allelic gene. Luttrell (1959) first pointed out that production of ascocarp was usually sparse and inconsistent with the same isolates under apparently identical conditions. We also experienced that different potential in ascocarp formation existed among different isolates. This phenomenon has been frequently observed in other Loculoascomycetous fungi such as *Cochliobolus miyabeanus* and other species of the genus *Cochliobolus.* The locule of pseudothecium was occupied by a mass of hyaline, filamentous pseudoparahyses extending downward from the inner well cells across the upper part of the ascocarp. Asci grew upward among the pseudoparaphyses from the base of the locule. The asci were cylindrical or clavate-cylindrical, with a short stipe. The walls were thick especially in the young asci. The asci were bitunicate type, with a thin outer wall that split at the apex, upon spore discharge, permitting expansion of the thick, and extensible inner wall. No asci were found with a full complement of 8 ascospores; the number of mature spores varied from 1 to 6; 2 or 4 were the most common (Fig. 4, A). Ascospores, fusiform, usually are 3 septa, few are more (Fig. 4, B). Leonard (1976) indicated that although mating *E. rostrata* ocourred at 20°C. the cultures required a preconditioning period of growth at low temperature before mating. Cultures recently transferred from storage at 4°C often formed ascocarps when paired in compatible combinations, but subcultures grown continuously at room temperature did not form ascocarps. On *E. turcicum,* we did not find the same requirement. Cultures for mating were usually incubated at room temperature without any period of low temperature treatment. Results of mating experiments also showed that some pairs of matings produced more ascocarps and some less. We believe that different potential in cross-fertility is prevailingly existed in different isolates as was always observed on *C. miyabeanus,* and Kolmer & Loenard (1985) deomonstrated that variation in fertility in *C. heterostrophus* was determined to be under the control of the perithecial gene locus and polygenic effect.

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玉米、貴黍煤紋病菌之比較研究

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本省高粱玉米煤紋病菌 *Exserohilum turcicum* (Pass) Leonard & Suggs 有兩種致病品系。從玉米煤紋病斑分離得到的菌株，只能在玉米葉上引起典型的煤紋病，而不能在貴黍葉引致典型的病斑。同樣的，從貴黍的菌株也不能感染玉米，只能在本身的寄主植物上形成典型的病斑。兩種品系菌株菌形態上幾乎都相同，只有在分生孢子梗的長度有差異，玉米株菌系的分生孢子梗比貴黍株菌系者長很多。但是貴黍株菌和玉米株菌系交配能形成子囊世代，產生成熟的子囊孢子。煤紋病菌的子囊世代爲 *Setosphaeria turcica* (Luttrell) Leonard & Suggs，煤紋病玉米株菌系在實驗室用的培養基上，如果蔬汁洋菜培養基，馬鈴薯煎汁液培養基以及麥芽抽出液培養基上培養，經幾次移植培養，往往發生退化現象，如菌絲生長異狀，往往朝空中卷曲，菌落甚或停止生長，失去孢子形成能力。我們發現菌株培養在含百分之一的洋菜培養基上，菌株恒能保持其原始活力，一旦移植到上述之培養基，往往生長發育良好，本菌分生孢子形成受持續光照的抑制，抑制有效波長在藍綠之間，貴黍菌株的分生孢子在貴黍葉表發芽後發芽管先端很快形成吸附器吸附在葉表面，但是在玉米葉表發芽者往往生成迷走於葉表之菌絲，鮮有形成吸附器。