

Pathogenicity and Host Range of *Acremonium lactucae* sp. nov., the Causal Agent of Leaf Brown Spot of Lettuce

Hsiu-Ju Lin¹, Chiu-Yuan Chien², and Jenn-Wen Huang^{3*}

1 Kaohsiung Branch Office, Bureau of Animal and Plant Health Inspection and Quarantine, Council of Agriculture, Executive Yuan, Kaohsiung 700, Taiwan

2 Institute of Biological Sciences, National Taiwan Normal University, Taipei, Taiwan.

3 Department of Plant Pathology, National Chung-Hsing University, Taichung, Taiwan.

* Corresponding author (Fax: +886-4-22851676; E-mail: jwhuang@dragon.nchu.edu.tw).

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ABSTRACT

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A new foliar disease of lettuce has been found in several organic farms in central Taiwan since 1996. *Acremonium lactucae* sp. nov. was consistently isolated from diseased tissues. Pathogenicity tests showed that the fungus is the causal organism of the disease. Among 27 plant species and 6 lettuce cultivars tested, only lettuce cultivars and tricolor chrysanthemum were susceptible to the pathogen.

Key words : lettuce, leaf brown spot, new disease, host ranges, genus *Acremonium*

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is an important vegetable crop used in organic cultivation in Taiwan. In the summer of 1996, lettuce plants in several organic farms at Yunlin county in central Taiwan exhibited a new foliar disease. Its initial symptoms consisted of small, circular to irregular yellowish-brown spot lesions, occurring on the minor and lateral veins of the lower leaves (Figures 1A-B). The disease is limited at the organic farming areas. Economic losses of the crop were estimated to average 12%, however, damage varied greatly ranging from 0 to 25% depending on cropping history and environment. A previously undescribed fungus was isolated consistently from symptomatic leaves. Description of the new disease and identification of the new pathogen are reported here. A preliminary report of this work has been published (18).

MATERIALS AND METHODS

Isolation and observation

Pieces (5 X 5 mm) of tissues obtained from the brown spot portions of lettuce leaves (Figures 1A-B) were surface sterilized with 1% sodium hypochlorite for 3 min and placed on 2% (w/v) water agar. Fungi growing from the tissues after 1 week incubation were isolated to malt extract agar (MEA).

For morphological observations, cultures were grown on 10 ml of MEA or potato-dextrose agar (PDA) in 9-cm plastic Petri dishes and incubated at 24°C in the dark for 7 days. The cultures were also incubated on MEA on glass slides⁽³⁾ for 7-10 days in daylight at 20-25°C. Materials for morphological studies and illustrations were mounted in lacto-phenol cotton blue. All photographs were taken with a light microscope (Leitz Orthoplan). For scanning electron microscopy⁽¹²⁾, samples of the fungus grown onto MEA for 7 days were prefixed in 2% glutaraldehyde-p-formaldehyde in 0.05M sodium cacodylate buffer at pH 7.2 for 24h at 4°C. The samples were then washed four times with a 0.01M phosphate buffer solution at pH 7.2 for 5 min. Samples were dehydrated using a graded series of ethanol. After dehydration, samples were critical-point dried (HCP-2, Hitachi Koki Co. Ltd., Tokyo, Japan) with liquid carbon dioxide as a transitional fluid. The dried materials were adhered onto aluminum specimen mounts with colloidal silver paste, and then sputter-coated (ion coater, IB-2M, Giko Engineering Co. Ltd., Japan) with gold (approximately 15nm thickness). The specimens were examined and photographed on a Hitachi S-570 scanning electron microscope (Hitachi, Co. Ltd., Tokyo, Japan) at 15kV. At least 30 samples were observed in this experiment.

Pathogenicity tests

Fungal isolates (AL-0818 and AL-0724) used for pathogenicity tests were grown on PDA slants for 14 days

under a 12-hr light/12-hr dark cycle. Conidia were washed from the cultures with deionized water and filtered through a sterilized double layer of cheesecloth. The conidial suspension was adjusted to a concentration of 1.5×10^5 conidia per ml with the aid of a hemacytometer. Lettuce seedlings (*Lactuca sativa* L. cv. improvement, 4-week-old) in pots were inoculated with this inoculum suspension using a hand-held plastic spray bottle and incubated in a humid chamber for 24 hr. The inoculated seedlings were then moved to a greenhouse. Plants sprayed with distilled water were used as the controls. All treated plants were examined for brown spots on the leaves 5-14 days after inoculation. The experiment was repeated twice using four to five plants each time.

Host range

Crop species routinely grown in the Hsilo area where lettuce leaf brown spot occurred, and various lettuce cultivars were tested for their susceptibility to the pathogen. Tested

plants included cabbage (*Brassica oleracea* L. var. *capitata*. L. cv. cavolo cappuccio cuore di bue), carrot (*Daucus carota* L. cv. hong tian), celery (*Apium graveolens* L. var. *dulce* Pers. cv. tall utah), Chinese kale (*Brassica alboglabra* Bailey. cv. round leaf and white flower), Chinese mustard (*Brassica chinensis* L. cv. san feng), edible amaranth (*Amaranthus tricolor* L. cv. white leaf), leaf mustard [*Brassica juncea* (L.) Czerniak cv. jie qing cai], pea (*Pisum sativum* L. cv. Taichung No. 11), radish (*Raphanus sativus* L. cv. xia feng No. 2), tomato [*Lycopersicon lycopersicum* (L.) Karst. ex. Farw. cv. zhu hui], water convolvulus (*Ipomoea aquatica* Forsk cv. bamboo leaf), calendula (*Calendula officinalis* Linn. cv. F-070), yellow sultan (*Centaurea moschata* L. cv. F-135), corn (*Zea mays* L. cv. mei zhen), tricolor chrysanthemum (*Chrysanthemum carinatum* Schousb. cv. F-140), garland chrysanthemum (*Chrysanthemum coronarium* L. cv. large leaf), yellow button (*Chrysanthemum multicaule* Dest. cv. F-145), endive (*Cichorium endivia* L. cv. V-100), common cosmos (*Cosmos bipinnatus* Cav. cv. F-185), orange

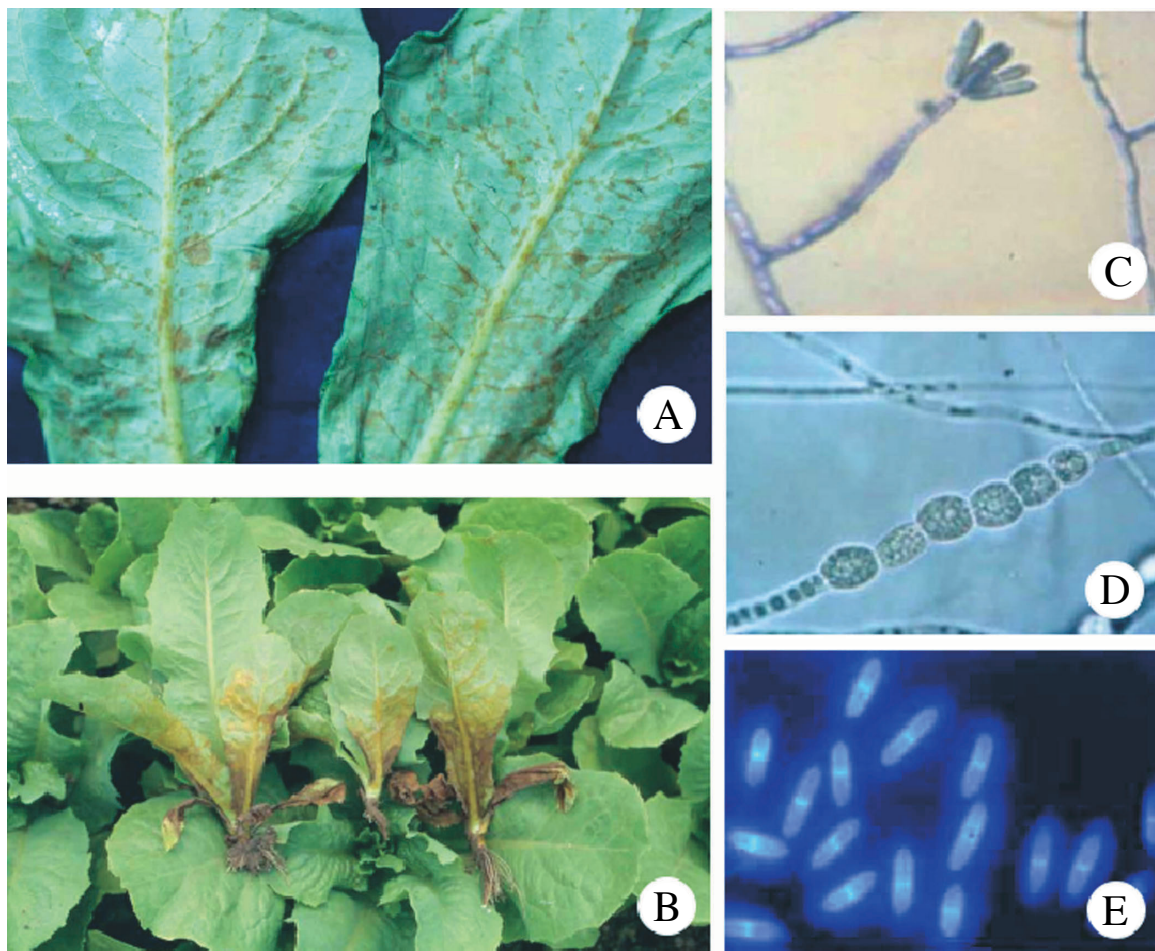


Figure 1. A-B, Symptoms of leaf brown spot of lettuce. A, small yellowish-brown lesions on the lower leaf abaxial surface of lettuce; B, severe necrosis of the lower leaves of diseased lettuce. C-E, Morphologies of *Acremonium lactucae*. C, conidia borne on a phialide stained with lacto-phenol cotton blue; D, chlamydospores in chain; E, two-celled conidia stained with calcofluor white M₂R⁽¹⁴⁾.

Table 1. Comparison of characteristics of *Acremonium lactucae* and other *Acremonium* spp. producing chlamydospores.

Fungus	Conidia		Chlamydospores Size (color) ^b	Colony (MEA) (mm diam./10 days) ^b
	Size (μ m) ^b	L/W ^c		
<i>A. lactucae</i> ^a	11.3-17.5 X 2.5-5.0	3.5-4.5	5.0-12.5 μ m (hyaline)	16.5-20
<i>A. minutisporum</i>	3.0-3.6 X 1.2-1.4	2.2-2.7	3.0-4.0 μ m (hyaline)	14-25
<i>A. kiliense</i>	3.1-5.8 X 1.0-1.6	2.6-4.5	4.0-8.0 μ m (hyaline)	18-23
<i>A. tubakii</i>	3.5-5.7 X 1.5-2.3	2.2-2.9	5-7 x 3.5 μ m (hyaline)	15-30
<i>A. falciforme</i>	7.8-10.0 X 2.7-3.3	2.8-3.3	5-10 μ m (dark brown)	10
<i>A. flavum</i>	5.0-6.0 X 1.7-2.5	—	5-7 μ m (hyaline)	13-17 ^d
<i>A. crotocinigenum</i>	4.4-7.4 X 1.6-2.8	2.0-3.1	5-8(-12) μ m (hyaline)	30-40
<i>A. recifei</i>	4.0-6.0(-7.5) X 1.3-2.0	2.7-3.6(-4.2)	3-5 μ m (hyaline)	12
<i>A. apii</i>	4.5-12 X 1.3-2.3	4.7-5.1	8-15 μ m (dark brown)	20-22
<i>A. restrictum</i>	3.0-4.3 X 1.6-2.4	1.3-2.5	4-8 μ m (dark brown)	6-8

^a Isolate (AL-0818) from lettuce (*Lactuca sativa* L.) at Yulin county in central Taiwan.

^b Information source from Gams, 1971⁽⁵⁾.

^c L/W:length/width.

^d mm diam. /6 days.

cosmos (*Cosmos sulphureus* Cav. cv. F-220), China aster [*Callistephus chinensis* (L.) Nees. cv. F-075], sunflower (*Helianthus annuus* L. cv. F-345), gazania (*Gazania splendens* hort. ex Moore. f. cv. F-305), creeping zinnia (*Sanvitalia procumbens* Lam cv. F-575), cineraria (*Senecio cruentus* DC. cv. quang hui-mixed), cape daisy [*Venidium fastuosum* (Jacq.) Stapf. cv. F-625], zinnia (*Zinnia violacea* Cav. cv. F-660), round leaf lettuce (*Lactuca sativa* L. cv. improvement), powder leaf lettuce (*Lactuca sativa* L. cv. ming feng No. 3), head lettuce (*Lactuca sativa* var. *capitata* L. cv. san yuan), curled lettuce (*Lactuca sativa* var. *crispa* L. cv. grand rapid), indian salad lettuce (*Lactuca indica* var. *indivisa* L. cv. small pointed leaf), and asparagus lettuce (*Lactuca sativa* var. *asparagina* L. cv. beseeming) Seedlings were grown in pots for four weeks and inoculated as described above. All test plants were examined for leaf symptoms 1-3 weeks after inoculation. The above experiment was repeated twice.

RESULTS

A fungus belonging to the genus *Acremonium* was consistently isolated from diseased tissues. It does not fit one of the recognized species^(1,6,7,8,9,10,15,16). A new species is,

therefore, described.

Acremonium lactucae Lin, Chien and Huang sp. nov. (Figures 1C-E)

Coloniae in agar malti (MEA), post 10 dies 24°C 16-20 mm diam. Mycelium tangerinum, reversum hyalinum. Hyphae vegetativae, tenuitunicatae, 2.5-2.8 μ m latae. Chlamydosporae post 14-20 dies abundantes, hyalinae globosae bis ovalis. Conidiophorae post dies sparsa, in mycelio aereo. Phialis singulae orthotropicae, raro ramosa, bis flexus, erectus, variabilis in longae, e 2 μ m ad 0.8 μ m sursum attenuatae. Conidiae, hyalinae, tenuitunicatae, singulatim in vel in mucilaginae. cylindriae bi-cellularis, atruncatae, levia 11.3-17.5 x 2.5-5.0 μ m. Teleomorphosis ignota.

Holotypus: Colonia exsiccata et, cultura viria AL-0818 isolata ex brunneus-punctatio folium, *Lactuca sativa* L., cultivar Hsilo, Yunlin in Taiwan, 1996.

Holotype: AL-0818(dried culture), National Museum of Natural Science (NMNS), No1 Kuan Chien Road, Taichung, Taiwan

Cultures from the holotype have been deposited at Cultural Collection Research Center, Food Industry Research and Development Institute (FIRDI), Hsinchu, Taiwan. Pure cultures and microscopic slides have also been deposited at

Table 2. Pathogenicity test of *Acremonium lactucae* isolates AL-0818 and AL-0724 in greenhouse tests.

Name of crop	Pathogenicity test ^a	
	AL-0818	AL-0724
Cabbage (<i>Brassica oleracea</i> L. var. <i>capitata</i> L.)	—	—
Carrot (<i>Daucus carota</i> L.)	—	—
Celery (<i>Apium graveolens</i> L. var. <i>dulce</i> Pers.)	—	—
Chinese kale (<i>Brassica alboglabra</i> Bailey.)	—	—
Chinese mustard (<i>Brassica chinensis</i> L.)	—	—
Corn (<i>Zea mays</i> L.)	—	—
Edible amaranth (<i>Amaranthus tricolor</i> L.)	—	—
Leaf mustard [<i>Brassica juncea</i> (L.) Czerniak]	—	—
Pea (<i>Pisum sativum</i> L.)	—	—
Radish (<i>Raphanus sativus</i> L.)	—	—
Tomato [<i>Lycopersicon lycopersicum</i> (L.) Karst. ex. Farw.]	—	—
Water convolvulus (<i>Ipomoea aquatica</i> Forsk.)	—	—
Calendula (<i>Calendula officinalis</i> Linn.)	—	—
Yellow-sultan (<i>Centaurea moschata</i> L.)	—	—
Tricolor chrysanthemum (<i>Chrysanthemum carinatum</i> Schousb.)	+	+
Garland chrysanthemum (<i>Chrysanthemum coronarium</i> L.)	—	—
Yellow button (<i>Chrysanthemum multicaule</i> Dest.)	—	—
Endive (<i>Cichorium endivia</i> L.)	—	—
Common Cosmos (<i>Cosmos bipinnatus</i> Cav.)	—	—
Orange cosmos [<i>Cosmos sulphureus</i> (L.) Cav.]	—	—
China aster (<i>Callistephus chinensis</i> Nees.)	—	—
Sunflower (<i>Helianthus annuus</i> L.)	—	—
Gazania (<i>Gazania splendens</i> hort. ex Moore. f.)	—	—
Creeping Zinnia (<i>Sanvitalia procumbens</i> Lam)	—	—
Cineraria (<i>Senecio cruentus</i> DC.)	—	—
Cape Daisy [<i>Venidium fastuosum</i> (Jacq.) Stapf.]	—	—
Zinnia (<i>Zinnia violacea</i> Cav.)	—	—
Round leaf Lettuce (<i>Lactuca sativa</i> L.)	+	+
Powder leaf Lettuce (<i>Lactuca sativa</i> L.)	+	+
Head lettuce (<i>Lactuca sativa</i> var. <i>capitata</i> L.)	+	+
Curled lettuce (<i>Lactuca sativa</i> var. <i>crispa</i> L.)	+	+
Indian salad lettuce (<i>Lactuca indica</i> var. <i>indivisa</i> L.)	+	+
Asparagus lettuce (<i>Lactuca sativa</i> var. <i>asparagina</i> L.)	+	+

^a + : Yes ; — : No

the Laboratory of Plant Disease Management, Department of Plant Pathology, National Chung-Hsing University, Taichung, Taiwan, R.O.C.

A. lactucae is a slow growing fungus, reaching on 16-20 mm in diameter on MEA and 20-26 mm on PDA for 10 days at 24°C. The optimum temperature for growth was 28°C. The fungus did not grow at either 8°C or 36°C. Colonies are pale orange to orange in color with slightly cotton-like aerial mycelia. Vegetative hyphae are hyaline, thin-walled, 2.5-2.8 μm wide. Phialides are simple or occasionally branched, straight to somewhat bent, erect on the hyphae, variable in length, and tapering from 2 μm near the base to 0.8 μm at the tip with imperceptible collarette and periclinal wall-thickening. Conidiophores usually consists of simple phialides (Figure 1C). Conidia 11.3-17.5 x 2.5-5.0 μm, borne singly but remaining in slimy heads are hyaline, two-celled, cylindrical, smooth-walled and untruncated, with length /

width ratio of 3.5-4.5 (Figure 1E). Chamydospores 5-12.5 μm diam, which appear after 14 days on MEA, are abundant, globose to oval, hyaline, terminal or intercalary, single or in chain (Figure 1D). Under SEM, conidium is one-celled in the initial development (Figure 2A) and chamydospores are smooth-walled (Figure 2B). Conidia of *A. lactucae* are larger than those formed by *Acremonium* species capable of producing chlamydospores (Table 1).

Symptoms

For lettuce, the initial symptoms consisted of small (less than 5 mm in diameter), circular to irregular, yellowish-brown to brown leaf spots, occurring on the minor and lateral veins of the lower leaves. These spots later enlarged and became elliptical to oblong in shape and brown in color. Although the lower leaf may show abundant infection, the higher leaves are rarely infected. Lesions are often so numerous that they coalesce to form areas extending several centimeters along

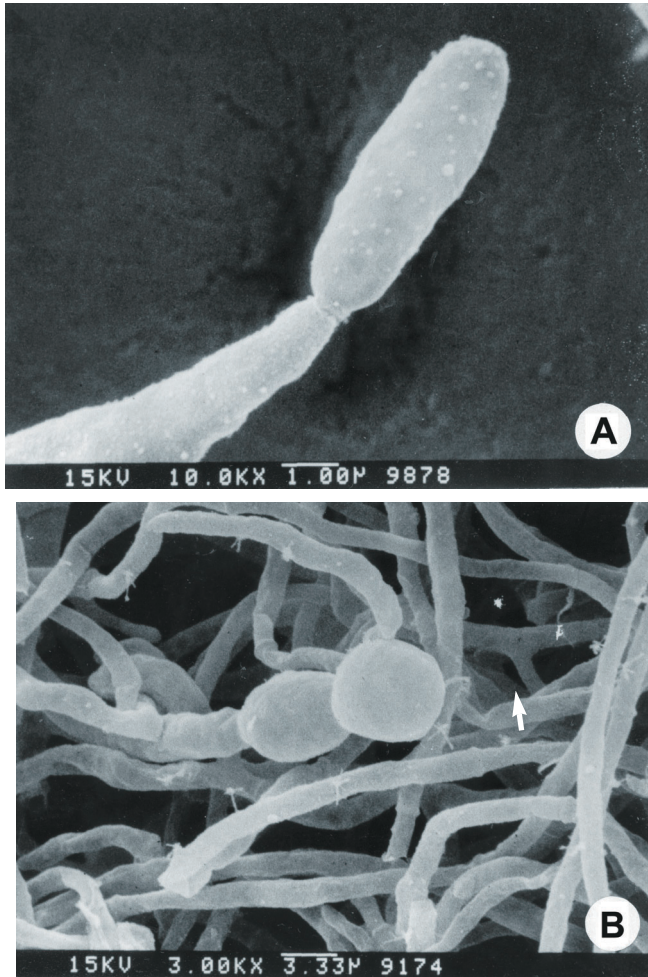


Figure 2. Scanning electron micrographs of *Acremonium lactucae*. A, conidial development; B, mycelia with chlamydospores (Arrowhead).

the leaf veins (Figure 1A). Infections may also result in a severe necrosis of the lower leaf (Figure 1B). The transverse sections of the affected veins show that this disease is confined primarily to the epidermal cells, although occasionally one or two layers of subepidermal cells may be invaded.

Pathogenicity and host range

Symptoms began to appear on all inoculated lettuce plants after 6 days. Inoculated plants with brown spots had the same symptoms as those seen in the field. Control plants did not show any disease symptoms. *A. lactucae* was recovered from lesions of the artificially infected plants. Results of host range tests showed that similar leaf spots developed only on six lettuce cultivars and tricolor chrysanthemum. Other inoculated plants remained symptomless (Table 2). The control plants remained free of the disease. This pathogen was re-isolated from leaf lesions on all the susceptible plants tested.

DISCUSSION

Acremonium is a large genus of morphologically simple hyphomycetes, representatives of which are commonly found in soil, on litter, and on various other substrate⁽⁵⁾. Several species of the genus *Acremonium* have been recognized as plant pathogens in the world. *A. strictum* was known to be the cause of seed rot, seeding blight, and ear mold disease of maize⁽¹³⁾, Shasta daisy vascular wilt⁽⁴⁾, and sorghum wilt⁽²⁾. The following diseases were also reported to be caused by species of *Acremonium*: brown spot of fig caused by *A. zonatum*⁽²⁰⁾; wilting of persimmon caused by *A. diospyri*⁽²¹⁾; brown spot of celery caused by *A. apii*⁽¹⁹⁾; quick decline of macadamia caused by *A. recifei*⁽¹⁷⁾, and melon *Acremonium* collapse caused by *A. cucurbitacearum*^(1,11). This study showed that the new species of *A. lactucae* is a casual pathogen of the severe brown spot in lettuce. This pathogen has a very narrow host range affecting only lettuce cultivars and tricolor chrysanthemum. The original source of inoculum in nature remains unknown. The pathogen was not found on other plants growing around the lettuce farms. Whether it was originated from soil remains to be investigated.

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摘 要

林秀儒¹、簡秋源²、黃振文³. 2004. 高苣褐斑病菌 *Acremonium lactucae* sp. nov. 的病原性與寄主範圍. 植病會刊 13: 91-96. (¹行政院農委會動植物防檢局高雄分局; ²台北市國立師範大學生物學研究所; ³台中市國立中興大學植物病理學系; 聯絡作者, 電子郵件: jwhuang@dragon.nchu.edu.tw, 傳真: +886-4-22851676)

公元 1996 年夏天於雲林縣西螺鎮有機蔬菜栽培區, 首次發現高苣植株的下位葉葉背出現許多細小圓形或不規則形的黃褐色斑點, 隨後病斑會相互融合, 造成葉背全面受害, 嚴重時會導致下位葉的壞疽現象。由罹病株分離到的 *Acremonium* sp. 菌株, 重新接種於高苣, 第六天後, 植株出現如同在田間相似的病徵, 隨後將具病原性的 *Acremonium* sp. 分別接種於不同栽培種的高苣、甘藍、芥菜、小白菜、甕菜、芥藍、莧菜、豌豆、蘿蔔、芹菜、西洋芹、番茄、胡蘿蔔、玉米、茼蒿、菊苣、翠菊、黃花矢車菊、春俏菊、花環菊、金盞花、黃波斯、向日葵、涼菊、小百日菊、大波斯、勳章菊、山衛菊與瓜葉菊等作物(包含菊科植物), 結果發現僅有高苣及花環菊有病徵出現。病原菌於麥芽抽出物瓊脂平板上生長緩慢, 菌落放射狀平貼於培養基表面, 呈淡橘色黏膜型, 其分生孢子圓筒狀, 兩端略尖, 雙胞, 無色透明, 大小為 11.25-17.5 × 2.5-5.0 μm, 長寬比(L/W)為 3.5-4.5; 分生孢子由瓶狀枝產生, 於瓶狀枝頂端聚集成假頭狀; 瓶狀枝單生直立或偶有分支, 沒有顏色, 長約 20-70 μm; 厚膜孢子呈圓形或橢圓形, 單細胞, 無色, 菌絲間生或頂生, 直徑大小為 5.0-12.5 μm; 利用掃描式電子顯微鏡觀察病原菌的產孢方式, 發現分生孢子自瓶狀枝內部產生。病原菌的最適生長溫度介於 24 與 28°C 間, 菌絲生長的最適酸鹼值為 5, 其分生孢子發芽與感染高苣的最適溫度均為 28°C。綜合上述病原菌的形態、產孢及生長特徵與其對高苣具致病性, 進而與國內外相關文獻比較, 高苣褐斑病的病原菌是一個新種, 故命名為 *Acremonium lactucae* sp. nov.。

關鍵詞: 高苣、高苣褐斑病、寄主範圍、新病害、頭孢菌屬。