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Scientific Notes

Postharvest Fruit Rot of Apples Caused by *Alternaria alternata* and *Stemphylium herbarum*

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Golden Delicious apples, both produced in Taiwan and imported from foreign countries, with rot symptoms were found in the markets in Taichung city in January 1994. Similar rot symptoms were also observed in several markets in the suburban area of Taichung city. Successive survey revealed that there were two types of rot symptoms on the diseased fruits. One of them was characterized by round and depressed lesion with brown to black brown color, dry and firm appearance on the surface of apples (Fig. 1) and rot tissues were spongy like. The other appeared as light to dark brown or black lesions around stem-end (Fig. 3), and had other characters, more or less similar to those of Alternaria rot. The two fruit rot diseases are often indistinguishable from each other unless the causal agents are isolated and identified. The fungus, *Alternaria alternata* (Fr.) Keissler, was found consistently associated with the former symptoms of rot (Fig. 2). Whereas a fungus identified as *Stemphylium herbarum* E. Simmons was consistently obtained from tissues of the latter symptom of rot on 2% water (WA) agar as well as on acidified potato dextrose agar (APDA) (Fig. 4).

Single spore cultures of *A. alternata* and *S. herbarum* isolated respectively from Delicious apples were used for cultural and morphological studies and pathogenicity test. Inoculum was prepared by culturing the fungus on PDA under constant fluorescent light, 100 to 400 F. C. at 25 C for 3 days. Apple fruits were wounded by a shallow, ting cut on fruit skin by sterilized scalpel and inoculated with either a 5-mm mycelium agar disc or a spore suspension containing approximately 10^5 conidia/ml. Inoculated areas on fruit were covered with moist cotton. Entire fruit was wrapped with a plastic bag immediately subsequent to inoculation and placed in a growth chamber with temperature between 20 and 24 C. Four replications of each treatment were conducted. Symptoms as described above were observed in all inoculated fruits after 3 days, but not in uninoculated fruits which were wounded only and served as control. Isolations from the inoculated fruits consistently yielded pure cultures of *A. alternata* and *S. herbarum* respectively. Whereas the controls were free of these two fungi. Koch's postulates were completed indicating that the two fungi were the causal agents of apple rots.

Following the morphological criteria described by Simmons (14), Neergaard (9), Wiltshire (16) and Booth *et al.* (1), the fungi causing apple rots were identified as *Alternaria alternata* and *Stemphylium herbarum*.

Alternaria alternata has simple or branched conidiophores, 18–50 × 3–6 μm; straight or curved, with one to several conidial scars. Conidia, 10–32.5 × 6.3–12.5 μm; averaging 21.2 × 9.5 μm, transverse septa, with one or two longitudinal septa (Fig. 5); formed in long, often branched chains, are golden brown and smooth; distinctly but not deeply constricted at major transverse septa.

Stemphylium herbarum has perfect stage *Pleospora herbarum* which produced perithecia abundantly on PDA culture. Perithecia are subemergent bodies, more or less sphaerical. Asci are bitunicate, cylindrical to clavate, 26–29 × 160–220 μm; each ascus produces 8 spores (Fig. 6). The ascospores are golden or brown, later turn darker, muriform, usually six or seven-septate, often constricted in the middle, with one to two longitudinal septa, 27.5–42.5 × 10–17.5 μm, averaging 32.2 × 13.6 μm. The conidia are muriform, with cross and longitudinal septa, often echinulate, yellow, turing black, 15.0–32.5 × 12.5–21.5 μm, averaging 24.3 × 15.3 μm (Fig. 7).

The effect of temperature and culture medium on the growth of *A. alternata* and *S. herbarum* were conducted. Four kinds of media, i.e., Czapek's agar (CA), oatmeal agar (OMA), V-8 juice agar (V-8A) and PDA, were used. Each agar plate was seeded with a 5-mm mycelial disc from the margin of an actively growing culture of *A. alternata* and *S. herbarum*, respectively. Four replicate plates of each kind of medium were maintained in darkness at 8 to 36 C with 4 C interval. Radial growth of the fungi was measured after 7 days. The results showed that the relative growth rate of *A. alternata* was greatest on CA and

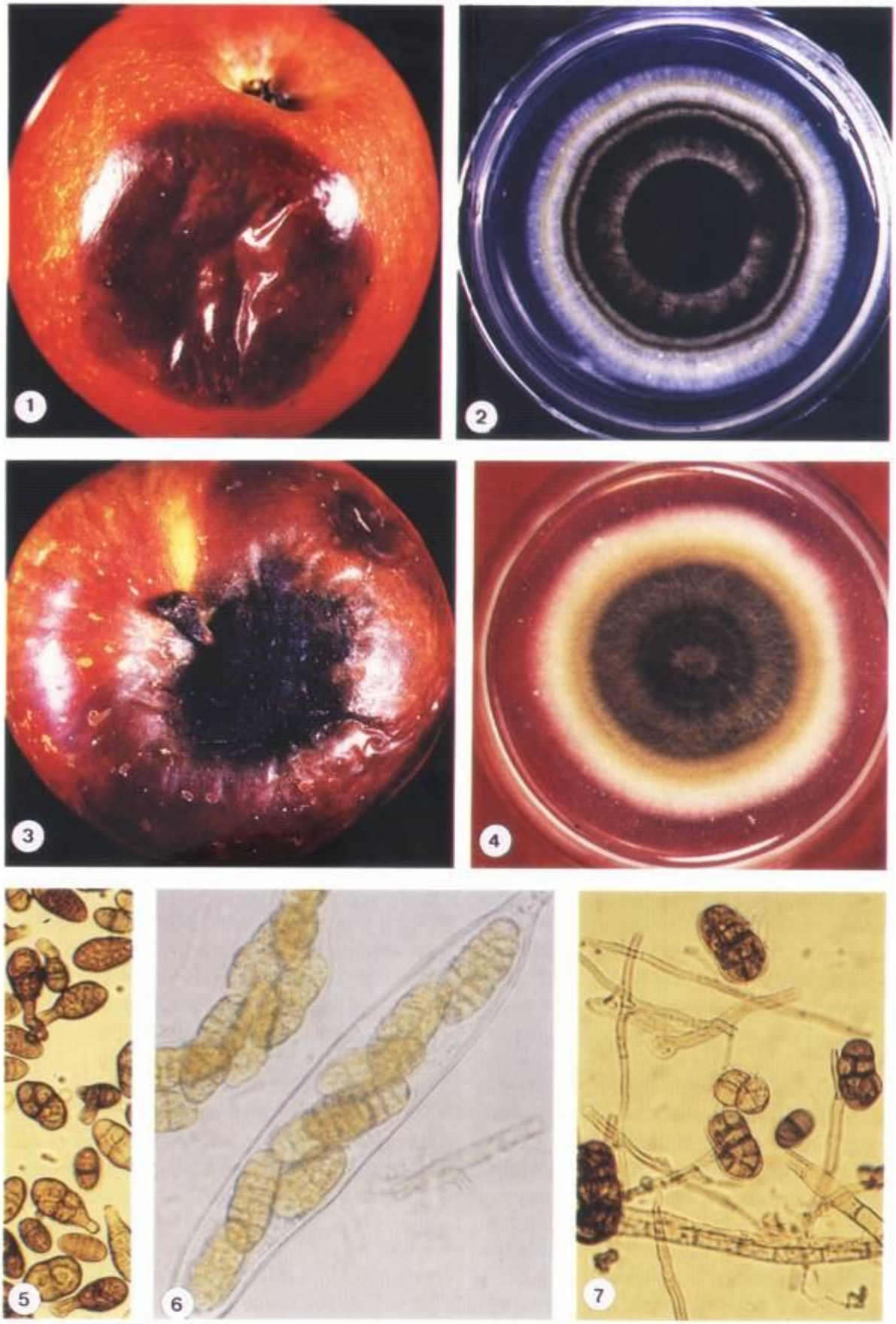

Fig. 1. Symptoms of Alternaria rot on apple caused by *Alternaria alternata*.
Fig. 2. Mycelial growth of *Alternaria alternata* on PDA from single conidium.
Fig. 3. Symptoms of Stemphylium rot of apple caused by *Stemphylium herbarum*.
Fig. 4. Mycelial growth of *Stemphylium herbarum* on PDA from single conidium.
Fig. 5. Conidia of *Alternaria alternata* from PDA culture.
Fig. 6. Perfect stage of *Stemphylium herbarum* from PDA culture. Ascus with eight ascospores.
Fig. 7. Conidia of *Stemphylium herbarum* from PDA culture.

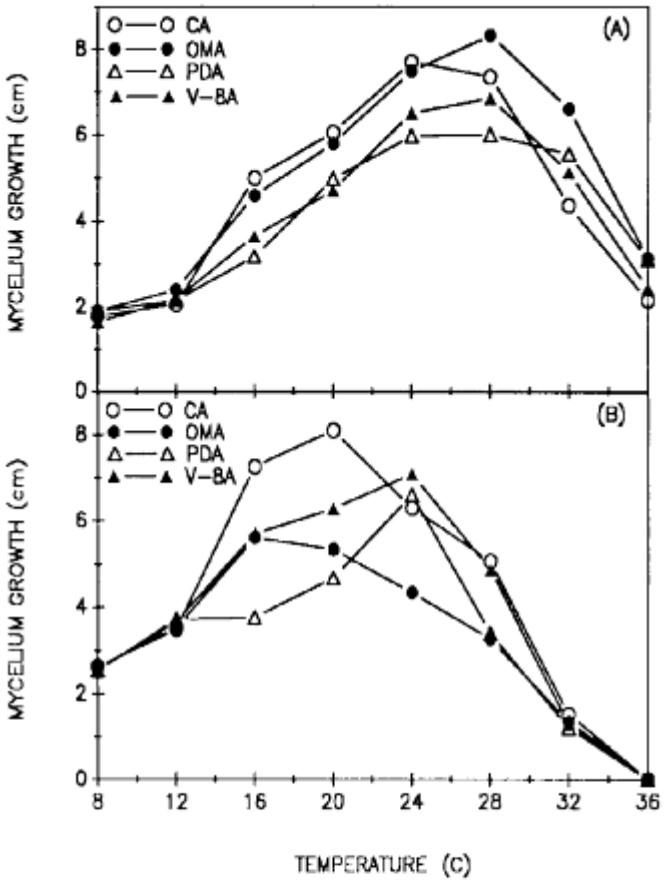

Fig. 8. Effect of culture media and temperature on growth of *Alternaria alternata* (A) and *Stemphylium herbarum* (B) 7 days after incubation. (CA = Czapek's agar; OMA = oatmeal agar; PDA = potato dextrose agar; V-8A = V-8 juice agar)

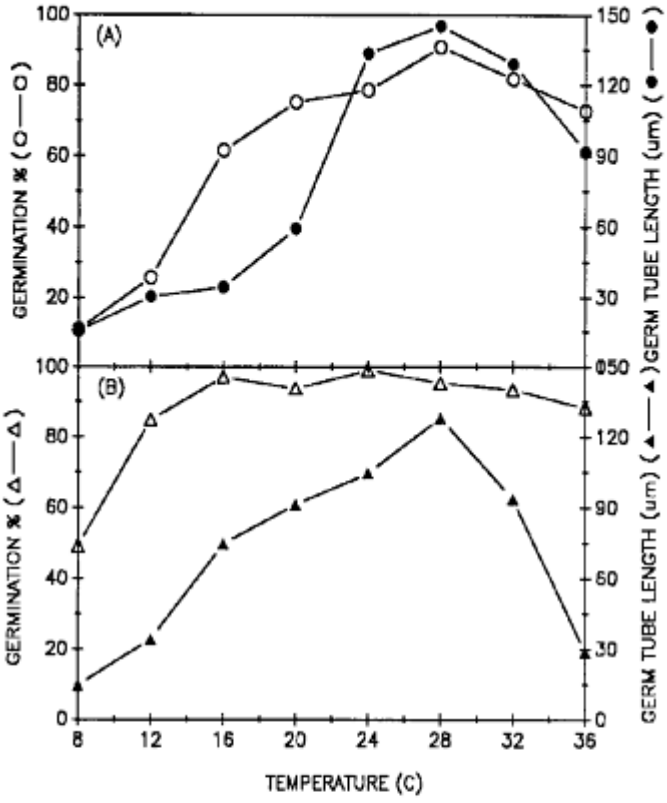

Fig. 9. Effect of temperature on conidial germination and germ tube elongation of *Alternaria alternata* (6 hr after incubation) (A) and *Stemphylium herbarum* (4 hr after incubation) (B).

OMA at 12–28 C (Fig. 8). For *S. herbarum*, CA and V-8A were more suitable for its growth (Fig. 8). The optimum temperatures for mycelial growth of *A. alternata* were 24–28 C (Fig. 8). For *S. herbarum*, the optimum temperatures for mycelial growth were 16–24 C (Fig. 8). Temperature effects on conidial germination and germ tube elongation were conducted by using conidial suspensions prepared from 10-day-old cultures of the fungi. Concentration of conidia were measured by a hemacytometer and adjusted to 10^5 conidia per milliliter. Two milliliters of the suspension were poured over the surface of 2% WA plate in 9-cm petri dishes and incubated at 8 to 36 C constant temperature with 4 C interval. Percent germination on four plates at each temperature was determined after 6 hr for *A. alternata* and after 4 hr for *S. herbarum*, respectively. The temperature suitable for conidial germination of *A. alternata* ranged from 16 to 32 C but the optimum was at 24–32 C (Fig. 9), which was also the optimum temperature for the germ tube elongation (Fig. 9). The temperature for conidial germination of *S. herbarum* showed a wide range between 8–36 C with the optimum around 16–28 C (Fig. 9). But germ tube elongation was shown best at 28 C (Fig. 9).

The genus *Alternaria* is ubiquitous and consists of plant-pathogenic and saprophytic species. The most common species, *A. alternata* (Fr.) Keissler, can infect a wide range of plants of different geographic regions (3). It can infect fruits before or after harvest (11,15). In addition to Alternaria rot of apple, *A. alternata* was recorded to cause several diseases in the apple orchards, including cork rot of Jonathan and Golden Delicious apples, ghost spot of young apple leaves, and moldy core and dry core rot of apples (6,11,15). It also causes stem decay of pear (11) and heart rot of mandarin (7).

Pleospora rot of apple has been reported in North America and Europe (2,6,8,10). Rose *et al.* (12) listed *S. congestum* Newtown, *S. congestum minor* Ruehle, and *P. fructicola* (Newton) Ruehle as minor important apple rots in the United States. *Pleospora pomorum* Horne is recorded as causal agent of apple spotting in England (5), and *P. fructicola* as causal agent of a firm brown rot of apple in the western United States (13). Neergaard (9) suggested that *P. pomorum* and *P. fructicola* be synonyms of *P. herbarum*. The high incidence of *Pleospora* that followed cold damage of fruit during storage suggests that *P. herbarum* can be a common saprophyte on the surface of apples (8). In Taiwan, this is the first report for the occurrence of postharvest fruit rot of Golden Delicious apples caused by *A. alternata* and *S. herbarum*.

Key words: Postharvest fruit rot of apple, Alternaria rot, Stemphylium rot.

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摘　　要

柯　勇、孫守恭、張朝芬．1994．貯藏期蘋果果實由 *Alternaria alternata* 和 *Stemphylium herbarum* 引起之腐爛病．植病會刊 3:128-131．(台中市　國立中興大學植病系)

1994年元月至三月間，筆者於台中市及市郊附近市場，經常發現蘋果腐爛病。罹病果實有兩種不同的病徵，一種爲果實罹病部位呈微凹之圓形，褐色至暗褐色，乾、硬表面皺縮之病斑，罹病組織呈海棉狀皺縮；另一種病徵呈淡褐至黑褐色病斑，常沿果蒂腐爛。這兩種病徵肉眼判別不易，須經病組織分離才可確定。前者之罹病組織分離、培養的病原菌是 *Alternaria alternata* (Fr.) Keissler；而後者則是 *Stemphylium herbarum* E. Simmons。以市售之蘋果進行接種試驗，無論是 *A. alternata* 或是 *S. herbarum* 兩種病原菌，分別以培養之菌絲塊或以孢子懸浮液行傷口接種，其病徵均與市場上所發現者相同，證實 *A. alternata* 和 *S. herbarum* 爲引起蘋果腐爛病之兩種病原菌。*A. alternata* 在 Czapek's 培養基和燕麥培養基上之菌絲生長最爲良好，其最適溫度在 24–28 C，而分生孢子發芽和發芽管生長最適溫度都在 24–32 C。而 *S. herbarum* 在 Czapek's 培養基和 V-8 培養基上生長最好，其生長適溫在 16–24 C，而分生孢子最適發芽溫度的範圍則較廣介於 12–36 C，但發芽管之生長適溫爲 28 C。在 PDA 培養基上，*Stemphylium herbarum* 極易產生它的有性世代 *Pleospora herbarum*。本報告之蘋果 Alternaria 及 Pleospora 腐爛病爲本省之新紀錄。

關鍵詞：蘋果貯藏期病害，Alternaria 腐爛病，Stemphylium 腐爛病。