MORPHOLOGY OF THE SWEET POTATO SCAB FUNGUS (*Sphaceloma batatas* Saw.)

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ABSTRACT

Specimens of sweet potato stem and foliage scab were examined microscopically and the causal fungus was cultured in agar media. Two types of conidia were observed, namely: the ovoid or oblong-elliptical designated as macroconidia with an average size of 6.99 x 12u and the minute and spherical referred to as microconidia with an average size of 3.22 x 1.97u. Some microconidia were borne on conidiophores while others lacked well-defined conidiophores whether on host tissues or in culture media. The origin of microconidia was not definitely established in this study. The macroconidia were made to germinate on various liquid media at different time periods. Germination was high in the sweet potato leaf exudate. The conidia enlarged and formed cross walls in 4 hr, formed germ tube and additional septa in 6 hr, branched in 48 hr and as branching became more developed, the hyphae thickened and became constricted in 68 hr.

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INTRODUCTION

Identification of a causal pathogen is a fundamental step towards controlling the disease it causes. In most cases, a fungus can be fully identified only after a thorough investigation of the various facets of its development. The unusual behavior of the sweet potato stem and foliage scab fungus in culture media and the presence of variable structures in both host and artificial media are not fully understood yet. A better grasp of its development is obtainable only through a careful consideration of its morphology in the various stages of growth.

The earliest available record known about the sweet potato stem and foliage scab was the reported 19 specimens collected from Formosa in 1910. Sawada identified the causal organism as *Sphaceloma batatas* in 1931 (Jenkins and Viegas, 1943). Goto (1937) reported that the

fungal structures found on a sweet potato stem canker from Amami Islands were about the same as those of the Formosa type given by Sawada. Jenkins and Viegas (1943) also described the pathogen from specimens collected from Campinas, Brazil. They detected, from the stem cankers, an ascomycetous fungus which they classified under the genus *Elsinoe*. They concluded that this was the perfect stage of *S. batatas*. In the Philippines, Divinagracia (1974) was the first to report on sweet potato stem and foliage scab disease, but his report did not include a detailed treatment of the morphology of the causal pathogen.

MATERIALS AND METHODS

Diseased Tissue. — Diseased tissue sections were prepared following the paraffin technique of Jensen (1962). To determine possible variations, the macroconidia and microconidia were collected from a number of sweet potato varieties which differed in their natural tolerances to scab.

Culture. — The fungus was cultured in agar media as previously described (Lao and Divinagracia, 1979). Bits of mycelia were extracted periodically from various portions of culture colonies and examined under the microscope to keep track of changes in their shape, color and other morphological structures.

Macroconidial Germination. — Infected parts of sweet potato plants were cut into pieces and incubated for 18-24 hr inside a humid chamber to promote production of conidia. Conidia scraped from the surface of lesions from Quezon 7 variety of sweet potato were placed onto drops of experimental liquid media on slides which were then placed inside Petri dishes and incubated at 25°C under constant light. Germination was observed by fixing the conidia on slides at 4, 6, 12, 24, 36, 48, 62 and 68 hr of incubation. The liquid media used were: sterilized distilled water as check; 2% sucrose, 0.2% sucrose; modified Fries medium, pure (Whiteside, 1975); modified Fries medium, 1: 7 (v/v); modified Fries medium, 1: 15 (v/v); sweet potato leaf decoction and sweet potato leaf exudate.

Sweet potato leaf exudate, one of the substrates used, was prepared by cutting and immersing 10 actively growing shoot apices in 10 ml distilled water for 1 to 2 hr. The water containing the exudate was sterilized in a pressure cooker at 20 psi for 15 min.

RESULTS AND DISCUSSION

The Fungus in Diseased Tissues.

It was found that the fungal hyphae in the colonized tissues were thick and septate (Fig. 1). Individual hyphal strands were hyaline to brown in unstained preparation.

Conidiophores produced in young lesions were hyaline, continuous or single-celled, cylindrical with tapered ends and slightly longer than the conidia (Fig. 1,

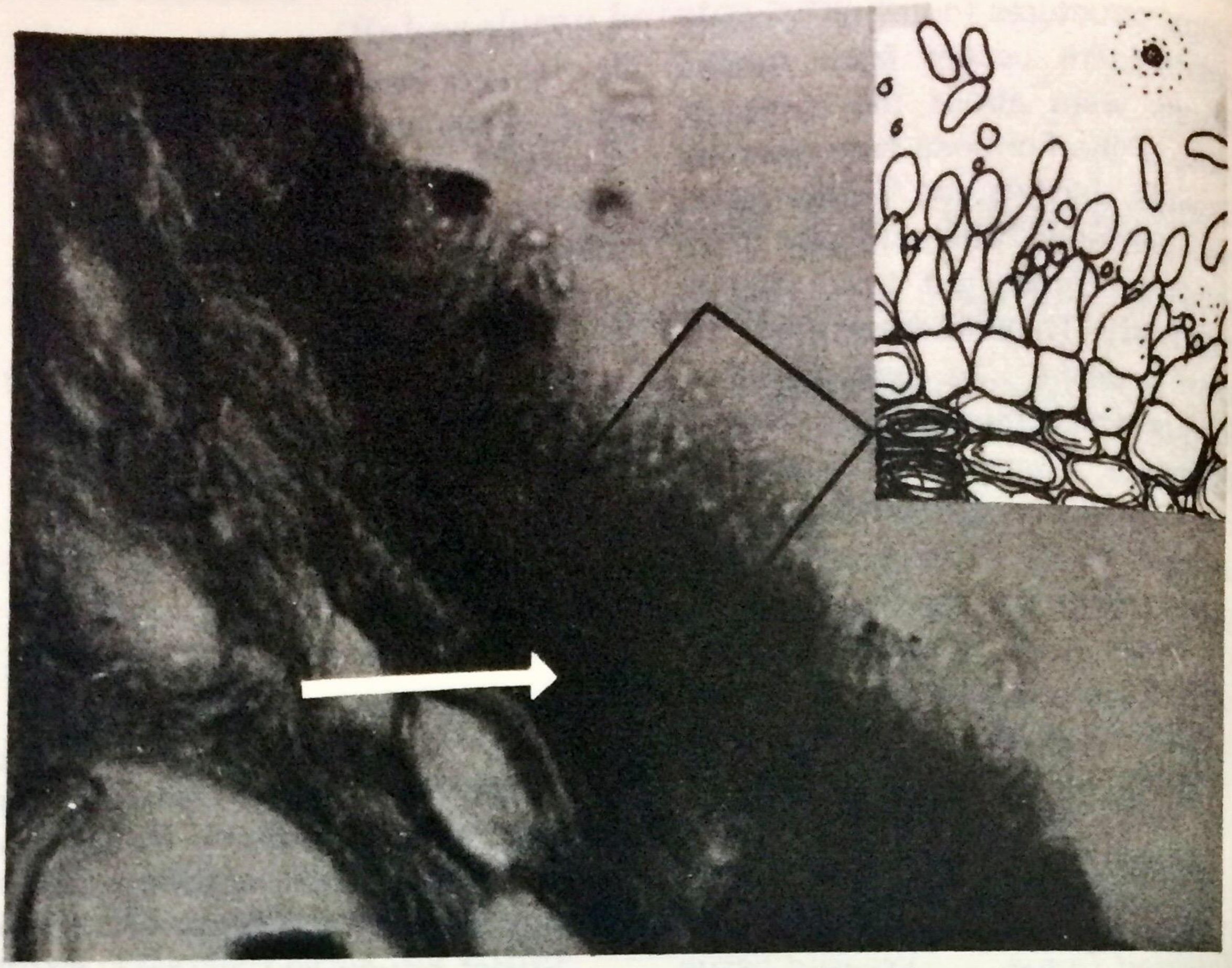


Fig. 1. Cross-section of an infected petiole showing conidia and mycelia (arrow) among collapsed epidermal cells and cortical parenchyma (X640). INSET: Drawing of cross-section through necrotic lesion showing microconidia, macroconidia and conidiophores.

inset). With age, they became colored and closely compacted forming palisade over the lesion. Acervuli were formed either on or beneath the epidermis. As measured from sections prepared from field specimens, the size of the conidiophores were 2.44 to 5.24u x 1.64 to 2.32u and the acervuli 8.52 to 36.0u in diameter. Whiteside (1975) in his experiments on *Elsinoe fawcetti*, observed that during the early stages of lesion development, the conidiophores were poorly defined and remained short where only hyaline conidia were produced.

Two types of conidia were produced by the fungus on the scab lesions. The kind present in greater number and more readily recognized were the hyaline and oblong-elliptical macroconidia (Fig. 2). These seemed covered with mucilaginous wall sometimes guttulated at either end. Occasionally, elongate, cylindrical and dark macroconidia, some of which were septated, were observed. Macroconidia produced from scab lesions ranged in size from 5.30 to 7.54u x 2.54 to 3.97u with an average of 6.41 to 3.40u. It was observed that most conidia were borne on conidiophores. In many cases, however, a large number of macroconidia were observed without well-defined coni-

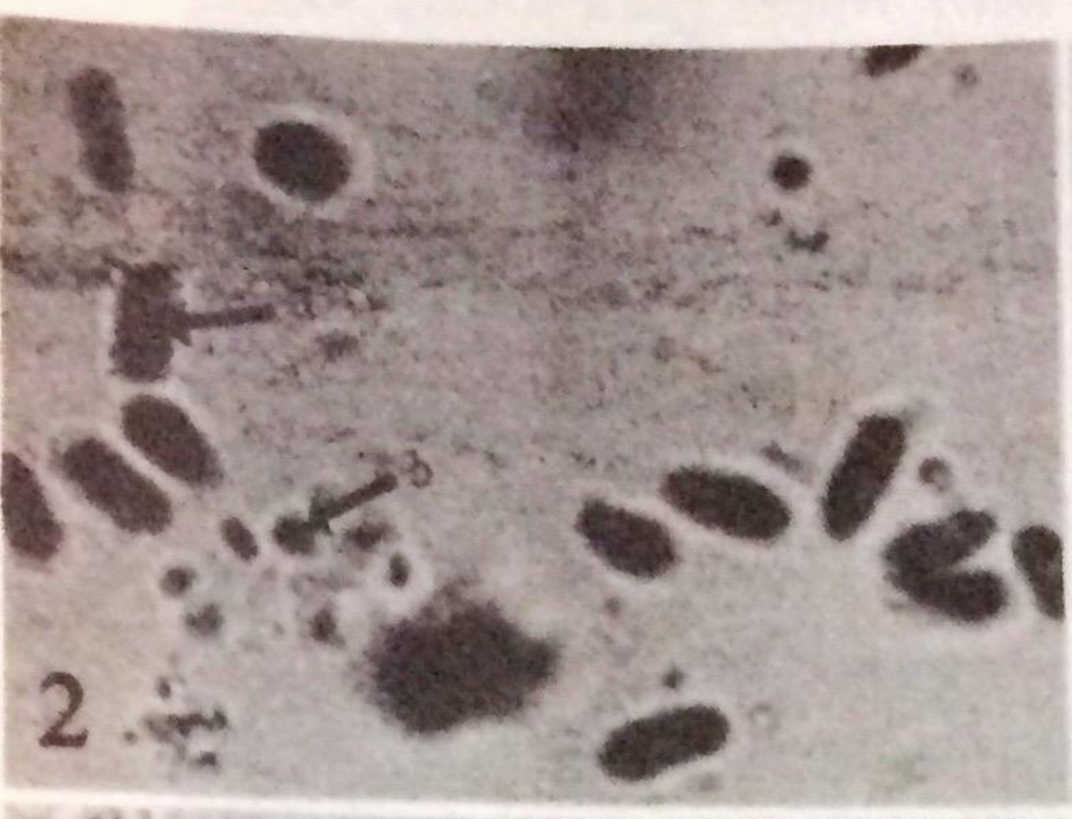
diophores, as confirmed by other workers. Jenkins (1931b) observed that the conidia of *Sphaceloma fawcetti* were sometimes produced on the sides of the conidiophores. Baines (1937) reported that the conidia of *S. menthae* were sessile upon the nonerumpent stromatic layer and no hyphae were observed that could be considered conidiophores. In addition, Kurata (1960) found in his microscopic examination of fresh leaf lesion of soybean scab that only a few conidia were seen attached to conidiophores.

The conidia on the scab lesions may develop outside the epidermis of the host tissues (Fig. 1). In other instances, the conidia may originate from the host's sub-epidermal layer. The rapidly increasing mass of conidia within the layer became erumpent. These structures were readily seen as solitary or confluent protrusions on the lesions.

Constantly in evidence and frequently associated with the macroconidia were minute and hyaline bodies provisionally called microconidia (Fig. 1 and 2). The bodies were extremely small and mostly spherical but some were slightly larger and ovoid or rod-like in shape. In some instances, the microconidia budded from each other or swelled slightly as they elongated. When in groups, they appeared as glistening masses aggregated with the conidia or scattered around the periphery of host tissues. They were found together with the macroconidia in the various stages of germination. The bodies were also seemingly covered with thick gelatinous wall like the macroconidia. These structures measured from 2.25 to 4.10u x 1.68 to 2.35u and averaged 3.22 x 1.97u.

The origin of microconidia observed in this work and reported by many workers from other host plants are not yet definitely known. Jenkins (1931b) remarked that the small glistening microconidia of *S. fawcetti* seen grouped about the pointed apices of conidiophores would have developed into larger elongate conidia under more favorable circumstances. Similarly, in this study numerous microconidia were constantly seen in the vicinity of the germinated macroconidia and in stromatic tissues. It was possible that upon exposure to favorable conditions, i.e., presence of moisture, the microconidia enlarged to form the elongate hyaline macroconidia as in *S. fawcetti.*

In the tissues dissected from the surface of a lesion from Quezon 7 variety of sweet potato, a group of structures believed to be ascocarp was observed (Fig. 3). These were the possible perfect stage. Each ascocarp (Fig. 4) contained several globose asci, each contained in a locule. The asci (Fig. 4) were of about the same size and age, and each contained seven or eight ascospores (Fig. 4). Ascospores were septated and a few were muriform. The sections, where the suspected perfect stage was found, were used as sources of conidia in germination studies.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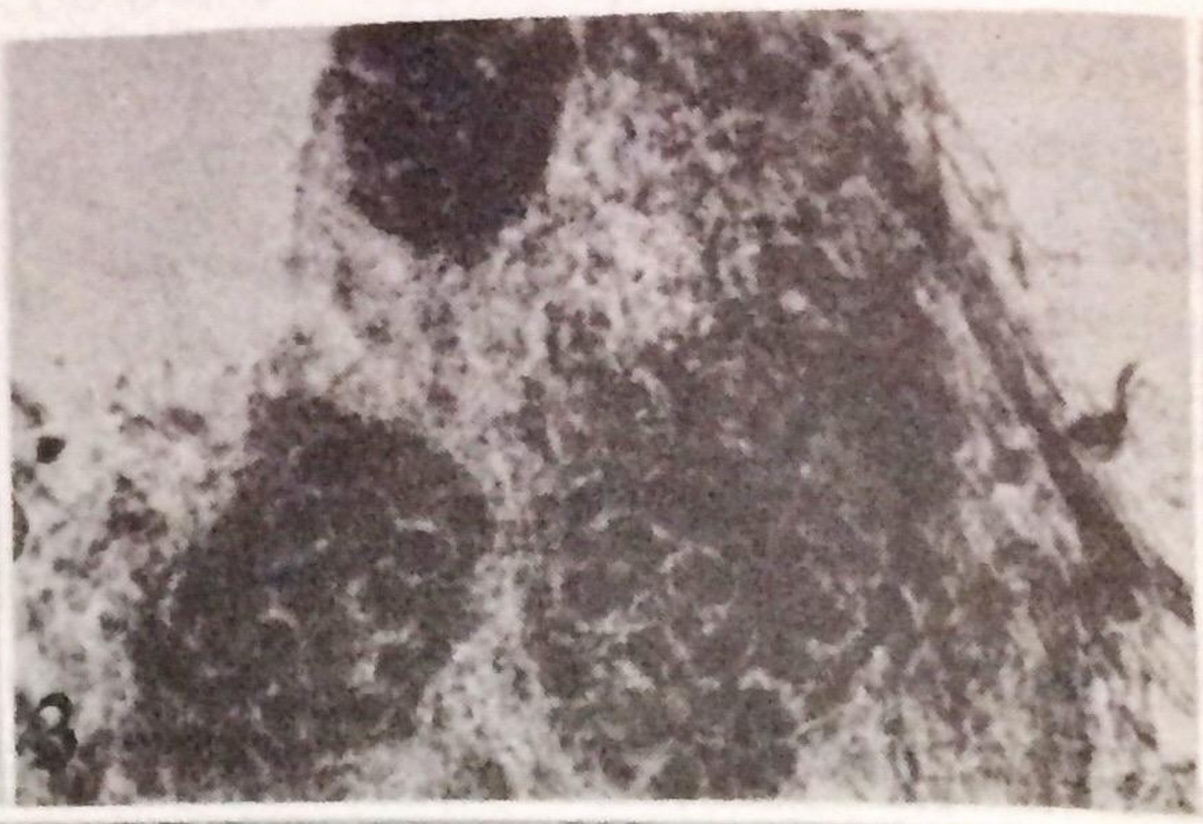
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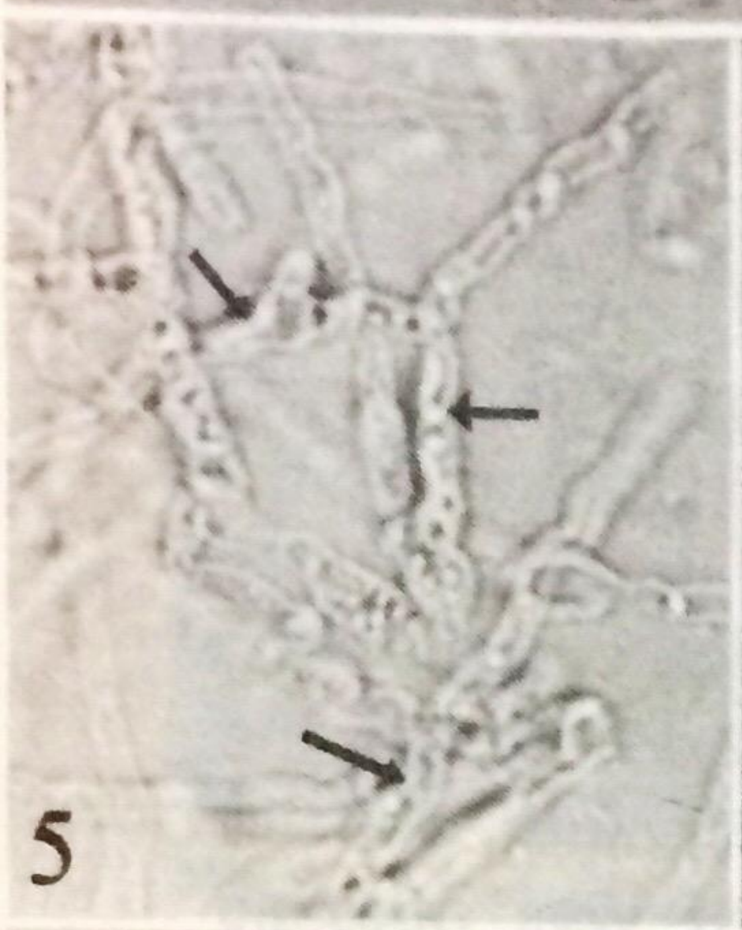
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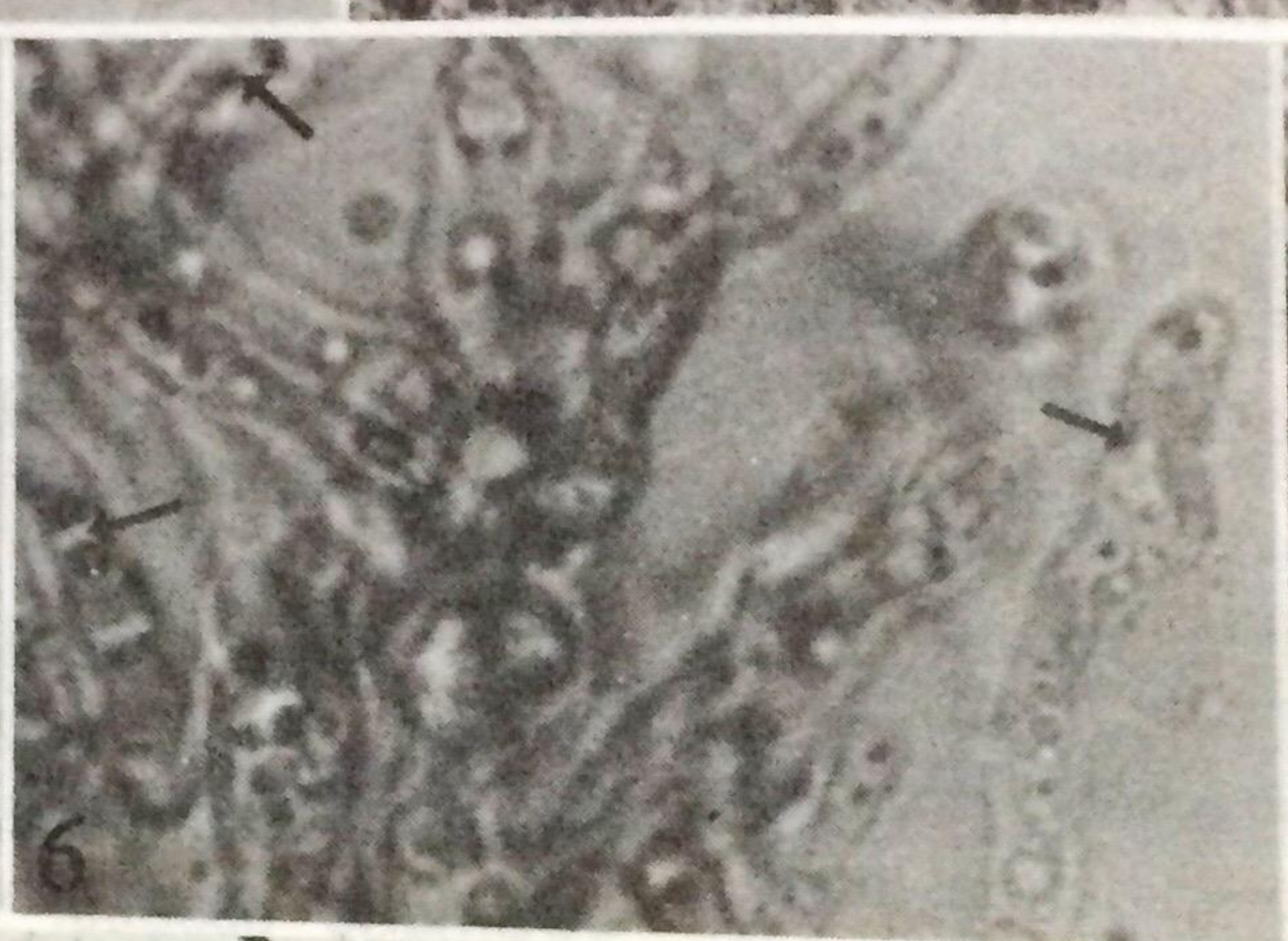
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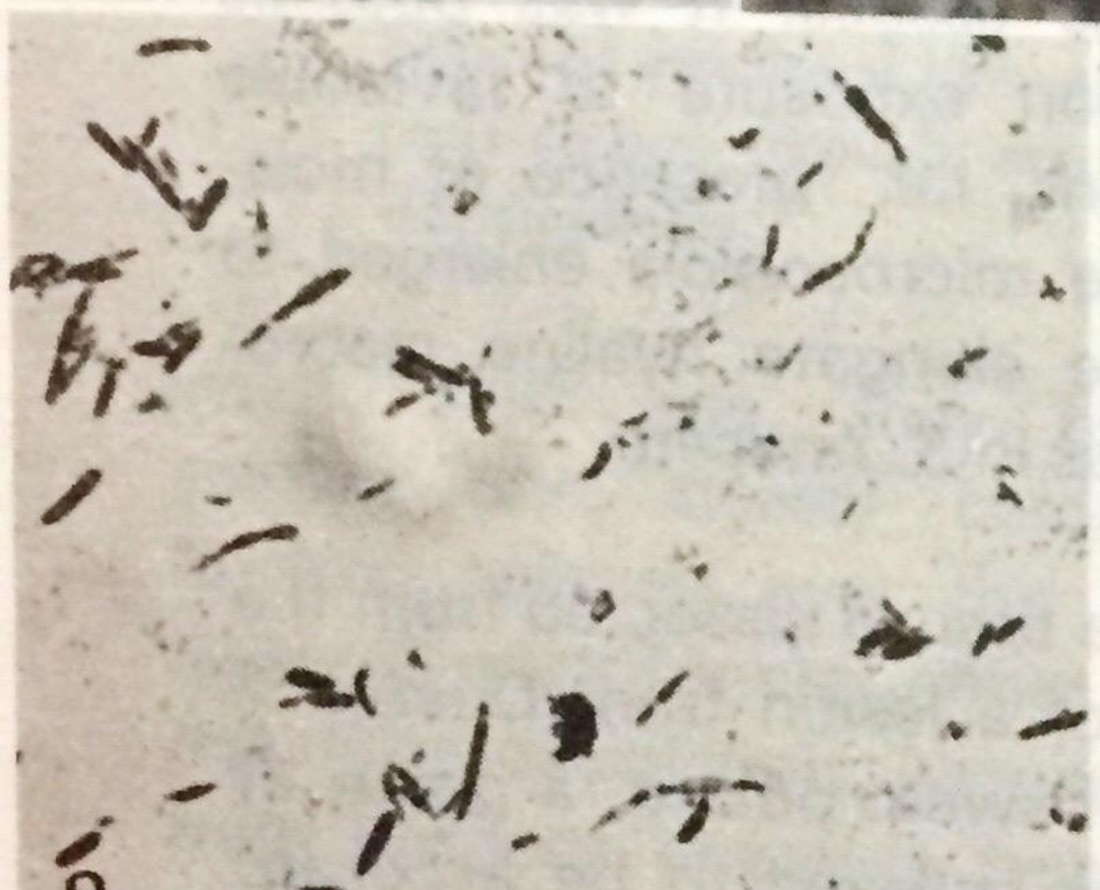
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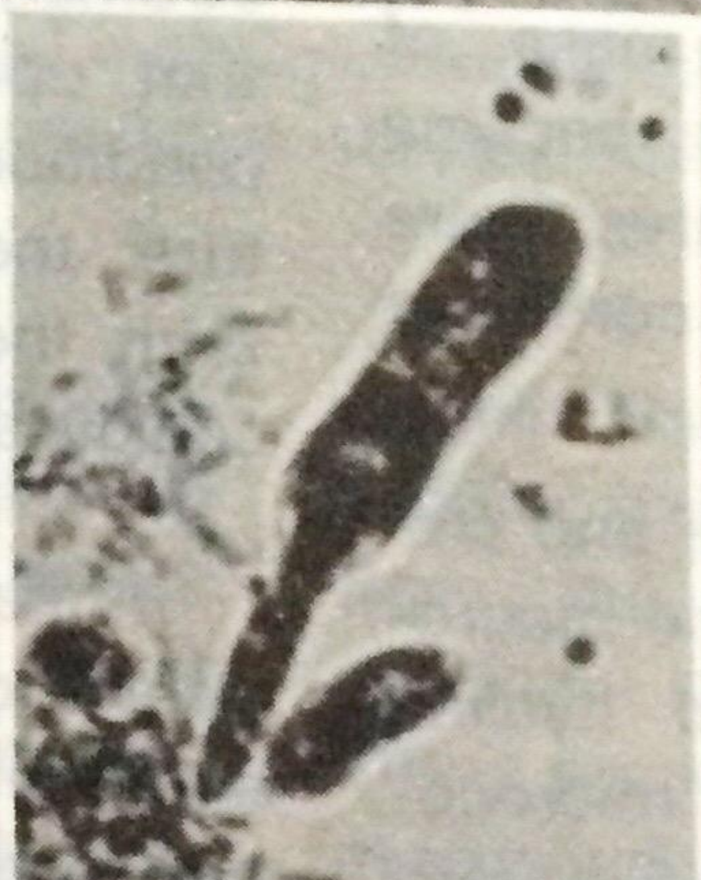
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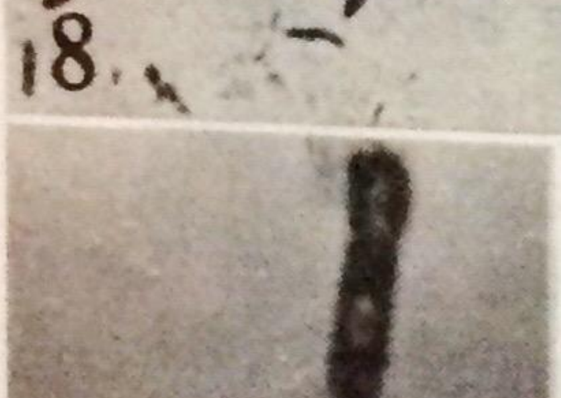

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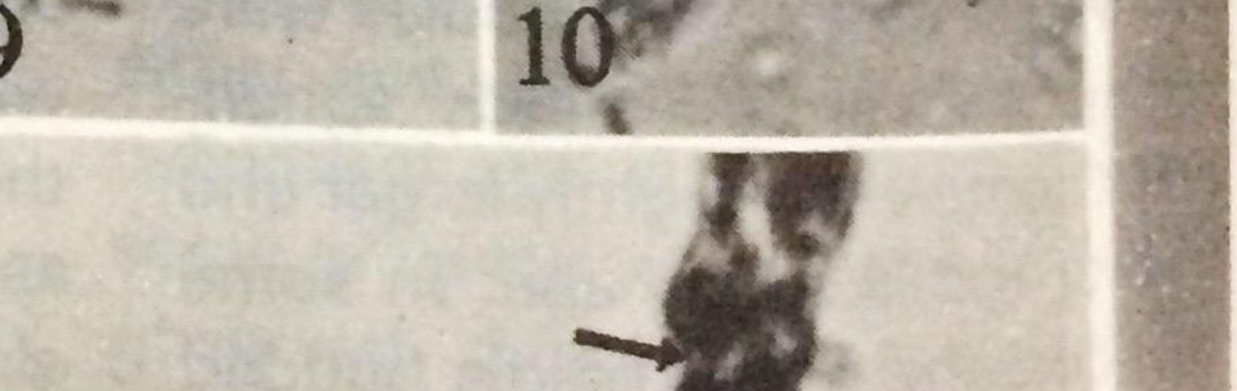
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The Fungus in Artificial Media.

The colonies were felty on the surface and were distinctly colored on each agar medium. These characteristics were also observed by Bitancourt and Jenkins (1937) on the cultures of *E. australis,* the sweet orange fruit scab. Other workers have observed the same color variation, such as Winston (1923) on the citrus scab fungus, Burkholder (1917) on anthracrose of raspberry pathogen, Jones (1924) on anthracrose of cane fruits, Jenkins (1931b) on *S. fawcetti,* Jenkins (1931a) on *E. canavaliae* of lima-bean scab and Baines (1938) on *S. menthae.* In this study, the colonies were vinaceous red on Leonian's, Hay's maroon on glycerin, cinnamon brown on onion, Molisch medium and potato dextrose, ox-blood red on malt, olivaceous black on sweet potato decoction, Dresden brown on yeast extract, orange-vinaceous on carrot, vinaceous-cinnamon on Czapeck's, chocolate brown on oatmeal and chestnut brown on prune agars. They were bound with distinctly different colors around the margins. The intensity and characteristic colors of the colonies remained even after 12 months of incubation.

The hyphae after two weeks were essentially continuous. At approximately three weeks, cross walls started to form and hyphae started becoming constricted at various points, usually at the juncture of septation. Constrictions were more readily seen on the sweet potato leaf decoction agar than on malt agar. They became more pronounced (Fig. 5 and 6) in subsequent weeks. Meanwhile, the hyphae anastomosed and gave rise to compact masses of thick-walled mycelia. The overall effect of these changes were convoluted, raised and compact colonies. The colonies retained their cultural characters even up to the 12th month with the hyphae greatly compressed into a thick mass of mycelium in the colonies and at the same time retaining its constricted nature. The

Fig. 2-14. Photomicrographs of conidia, ascocarps, hyphae and germinating conidia of *Sphaceloma batatas*. 2) Conidia from sweet potato variety 'C200-1'. (a) unicellular macroconidium, (b) microconidia (X1600). 3) Group of ascocraps (X160) 4) An ascocarp with (a) asci and (b) ascospores (X640). 5-6) Fungal mycelia from 21-week old agar cultures showing hyphal constrictions (arrows) (5, X640 , 6, X1600). 7) Macroconidia produced from a microcolony (X640). 8-14) Stages of macroconidial germination in sweet potato leaf exudate showing, 8) germ tube formation after 4 hr, (X160), 9) germ tube on one end after 6 hr, (X1600), 10) additional septa in 12 hr, (X1600), 11) developing hypha from the original cell (arrow) in 24 hr, (X1600), 12) two developed branches (a) near original segment (b) after 48 hr (X1600), 13) branching from the germinated conidial cell after 68 hr (X1600), 14) hyphal fragments 68 hr after germination showing constrictions (arrows) (X1600).

perfect stage of the fungus was not observed even in one-year old cultures.

The two types of conidia associated with the fungus in the host tissues were also observed in artificial cultures. By following the method of Whiteside (1975), the isolated fungus was induced to produce the ovoid or oblong-elliptical hyaline macroconidia (Fig. 7). The macroconidia were somewhat darker in color and also seemingly covered with mucilaginous substance. They were attached terminally and on the sides of hyphae in microcolonies. The size of the macroconidia from 16-day old malt agar culture, of isolate Bentong #2, ranged from 5.88 x 2.65u to 7.85 x 3.62u and averaged 6.99 x 3.12u. These were nearly similar with macroconidia from the host.

Macroconidia from four-week old cultures on malt agar at different hydrogen-ion concentrations appeared swollen and bigger than those from 16-day old malt agar culture. Their sizes at different hydrogen-ion concentrations are shown in Table 1. There was no marked difference in the size of the spores at various levels.

Microconidia resembled those produced in the plant tissues, which were often minute and spherical but occasionally ovoid or slightly elongated. The ovoid or elongated types were readily seen in the slide cultures in the germination studies using Fries medium. They budded readily in the same medium as in the leaf exudates. Similarly, they appeared to be coated with thick

Table 1. Average size of macroconidia from different hydrogen-ion concentrations of the medium.

pH of Medium	Size (u) [1]
5.5	**6.43 x 3.33**
6.0	**6.90 x 3.18**
6.5	**7.60 x 3.50**
7.0	**7.36 x 3.20**
7.5	**7.31 x 3.34**
8.0	**7.48 x 3.30**
8.5	**7.27 x 3.43**

[1] Average of 40 macroconidia.

gelatinous wall. They were as big as the microconidia in the host tissues.

Thus, some of the macroconidia scattered in the vicinity of microcolonies and those found in about two-week old agar cultures in this study probably have arisen from microconidia. It was noted that both macroconidia and microconidia were always present on the lesion surfaces especially on mature ones, while in agar media they could be seen at certain periods only. This was possibly due to the continuous supply of nutrients in the host tissues while such nutrients became depleted in artificial media.

The presence of macroconidia and microconidia together in scab lesions and in colonies in culture media was further supported by other findings. Bitancourt and Jenkins (1937) reported that the bodies interpreted as microconidia were usually present on the surface of the acervuli of *E. australis*. Kurata (1960) stated that the spores of *S. glycines* were either hyaline, spherical microconidia or ovoid to elliptical

pale-colored macroconidia. He noted that the two types of conidia intermingled together in masses, covering the entire surface of the lesion. Jenkins (1931b) considered the three types of conidia, namely, small spherical conidia, ovoid elliptical hyaline conidia and ovoid elliptical colored conidia, to be homologous or merely represented older or younger forms.

Under field conditions, the microconidia and macroconidia may cover the surfaces of sweet potato scab lesions. Frequently, the conidial fructifications were produced within the stromatic layer in the epidermis and sub-epidermis. With the onset of warm weather, the superficial fructifications would probably dry up but the latent hyphae and conidia embedded within the colonized tissues could serve as hold-over structures of the scab fungus. During rainy days when relative humidity is high, heavy scab infection would ensue due to renewed growth of the pathogen.

Macroconidial Germination.

Germination hardly took place in the other test media except in the sweet potato leaf exudate. The germination in this substrate was quite variable but uniformly high. Macroconidia enlarged and formed cross walls in 4 hr (Fig. 8). In 6 hr, some macroconidia formed germ tube on one end (Fig. 9). Others swelled and continued to form septa in 12 hr (Fig. 10). In 24 hr, hyphal growth was considerably longer than the macroconidium itself and, in some cases, a new germ tube might develop at the other end of the spore (Fig. 11). After 48 hr, branching started to occur near the original conidial cell (Fig. 12). In 68 hr, branching became more developed, the hyphae thickened and became constricted and branched in all directions (Fig. 13 and 14). Other morphological changes occurred concurrent with the enlargement of the conidia, septa formation and branching of the hyphae. Vacuoles developed as the cytoplasm moved to the advancing hyphal segments. These vacuoles were prominent in the various phases of growth.

The finding that the conidia of *S. batatas* were produced and germinated in relatively short periods of wetting was similar to the report of Whiteside (1975) in his experiments on *E. fawcetti.* Macroconidia were produced in microcolonies using Fries medium after 2-3 hr of incubation at 25°C and germinated after 4 hr of wetting in sweet potato leaf exudates. Yamada (1901) reported that conidia could be formed in 1.5-2.0 hr on scab lesions. Thus, the conidia embedded in the stromatic tissues could be induced to germinate and start infection when rain or other sources of moisture were made available in the field.

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