Ecological Role of Basidiospores of *Thanatephorus cucumeris* (Frank) Donk in the Incidence of Foliage Blight of Sugar Beets in Japan

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Introduction

Although *Thanatephorus cucumeris* (Frank) Donk, a telemorph of *Rhizoctonia solani* Kühn, is commonly recognized as a soil-borne root infecting fungi, it occasionally causes aerial diseases such as sheath blight, web blight or foliage blight in several crops[1,16,17]. These aerial diseases are mainly caused either by hyphae (or sclerotia) in soil or by basidiospores developed on soil and plant surfaces. The basidiospore infection however has remained an unsolved problem for a long time, especially in sugar beets in Japan. The foliage blight of sugar beets caused by *T. cucumeris* was first found in Hokkaido, Japan, at the beginning of 1950s[16], and since then it has widely spread in most parts of Hokkaido. The severity of the disease has shown great variations from year to year. In wet and hot midsummer, the disease spreads out very rapidly.

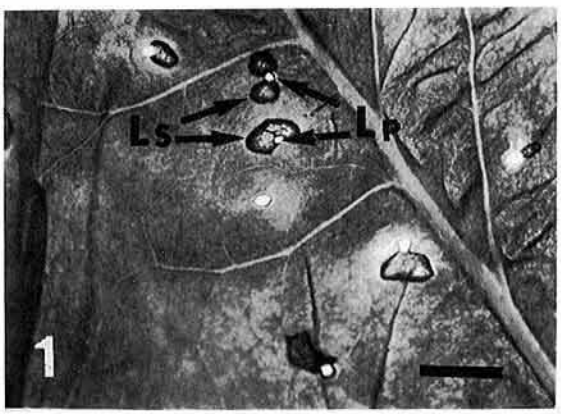
This paper presents results of the experiments on the spore infection and the significance of the perfect stage in the disease cycle of foliage blight of sugar beets.

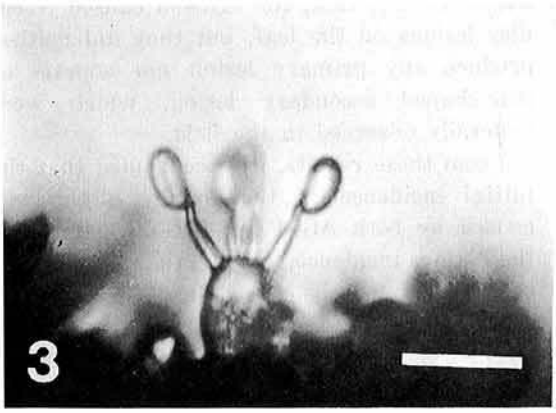
Symptoms

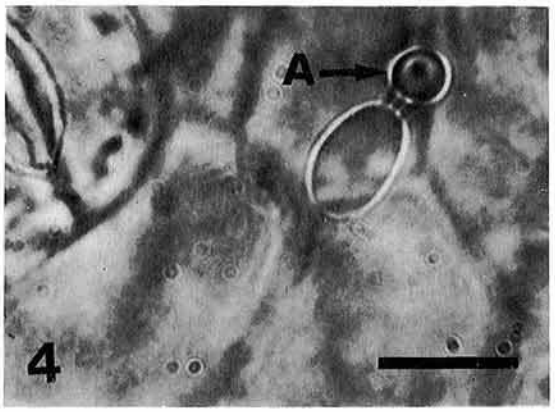
The signs of the foliage blight vary from dark green to brown in color with lesions of various sizes and shapes, which are distinguished by the following two kinds of lesions:

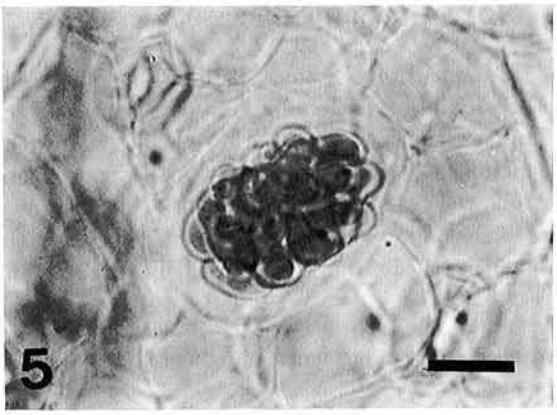
One is a primary lesion that shows an appearance of small, or 1 mm diameter, circular necrotic spots surrounded by a brown border (Plate 1–1 & –6), mostly on mature or taller leaves 30 cm above the soil surface, and no spot enlarges beyond that size. Under the low temperature or dry weather conditions, old primary lesions are frequently perforated in their middle part. Primary lesions are generally accompanied by secondary and/or larger lesions under the field conditions.

The other is a secondary lesion that is dark green to brown with circular, zonate, star- or irregular-shaped necrosis, from 3–5 mm wide to more than half a leaf in lesion size (Plate 1–2). The healthy green areas are generally scattered in the enlarged lesion. Most of the initial symptoms take place on healthy tissues adjacent to the primary lesions on a mature or taller leaf, but a few of them are seen on the unfolded young leaves or on the oldest leaves of outer whorls, where no primary lesions exist. The larger lesions which show typical blighting symptoms, as often called large-sized lesions, are included in the category of secondary lesion that is caused by hyphal infection. The greyish-white growing hyphae, and the powdery-white hymenia are sometimes observed with the naked eye on the ventral side of diseased leaves.









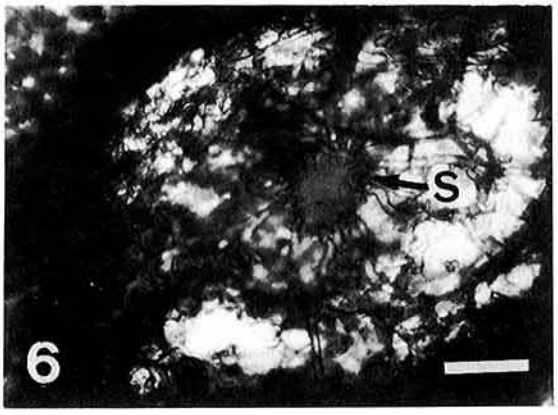


Plate 1-1: Primary lesion (Lp) and secondary lesion (Ls) in the field. Bar: 10 mm.
-2: Secondary lesion showing zonate shaped necrosis in the field.
-3: The basidial stage produced by AG-2-2 of *T. cucumeris* on the soil surface. Bar: 20 μm.
-4: Germinated basidiospore forming appressorium (A) on the leaf. Bar: 10 μm.
-5: Stroma-like body produced in the infected epidermal cell at 3 to 4 days after inoculation. Bar: 20 μm.
-6: Primary lesions. Note stroma-like body (S) in the center, chlorosis in the middle zone and browning in the outer zone of the lesion. Bar: 100 μm.

Anastomosis group of isolates of *T. cucumeris*[12]

Six hundred and fifty-three isolates of *T. cucumeris* were obtained from the blighted leaves of sugar beets which were collected from 52 fields in various parts of Hokkaido during the period early August to early October each in 1973–1975. Anastomosis group (AG) of these isolates were determined by anastomosing with standard isolates of each AG designated by Ogoshi[13].

All the isolates were classified in the following two anastomosis groups: AG–1 (web blight type) and AG–2–2 (root rot type) (Table 1). The isolation frequency of the two anastomosis groups greatly varied with the time of collection and the growth stage of sugar beet leaf. Early in August when the occurrence of foliage blight disease was light, most of the isolates from the lesions on young or unfolded leaves belonged to AG–1. while three-quarters of them from the taller or mature leaves were placed in AG–2–2. In the case of the oldest leaves near the soil surface, more isolates of AG–1 were obtained than those of AG–2–2. On the other hand, during the period late August to early September, when the foliage blight disease was severe, the isolates of AG–2–2 counted over 95% of the isolates obtained. In this case, however, no difference was seen among the stages of sugar beet leaves in respect to the frequency of isolations between AG–1 and AG–2–2.

As for the relationships between symptoms and anastomosis groups, most isolates obtained from the primary lesions and the secondary lesions as well as from the large-sized lesions bearing fruit bodies, were placed in the group of AG–2–2. On the contrary, a majority of the isolates from the irregular secondary lesions developed on the unfolded young leaves under light incidence of the disease were grouped in AG–1.

In the inoculation experiment on mycelia under hot and humid conditions in a vinyl house, all the foliar isolates of AG–1 and AG–2–2 tested caused foliage blight of sugar beets. In this case, the mycelia caused irregular lesions on the leaf, but they did neither produce any primary lesion nor zonate- or star-shaped secondary lesion, which were generally observed in the field.

From these results, it is concluded that the initial incidence of the foliage blight was caused by both AG–1 and AG–2–2, but that the later incidence, when the blight was severe, was mainly caused by AG–2–2. It is likely that basidiospores of *T. cucumeris* AG–2–2 severely caused the foliage blight on taller leaves in a short period.

Infection and disease incidence

As mentioned above, primary lesions could be found occasionally on the middle-aged taller leaves even in the period of initial incidence of foliage blight of sugar beets, followed by a rapid prevalence of disease over a field. These observations indicate that the primary lesion may be caused by a dispersing

Table 1. Anastomosis group *Thanatephorus cucumeris* in the incidence of foliage blight of sugar beets in fields in Japan

Stage of disease occurrence	No. of fields observed	Number of isolates obtained from each stage of leaf					
		Young aged leaf		Middle aged leaf		Old aged leaf	
		AG–1	AG–2–2	AG–1	AG–2–2	AG–1	AG–2–2
Initial[a]	13	42 (95.5%)	2 (4.5%)	35 (28.7%)	87 (71.3%)	29 (74.4%)	10 (25.6%)
Later[b]	16	1 (4.0%)	24 (96.0%)	10 (3.9%)	248 (96.1%)	2 (11.8%)	15 (88.2%)

a): Early August. b): Late August to early September.

basidiospore from hymenium on soil and on diseased plant surface as well, but not by hyphae growing out from soil surface or infected soil particles sprashing from there.

In the past, basidiospore infections of *T. cucumeris* have been reported in reference to several aerial diseases[2,4-9,14,15]. However, little is known about the infection process and the mechanism of prevalence of foliage blight of sugar beets. In the following sections, some results relating to foliage blight of sugar beets are presented, including process of lesion development and prevalence of disease, some factors influencing on spore germination, and survival of pathogen in soil.

1) Infection and lesion development[10]

Sporulation of *T. cucumeris* was induced in culture plates after being covered by soil after the Ogoshi's soil method (Plate 1-3)[13]. Discharged basidiospores dropped on sugar beet leaves by placing basidia clusters of *T. cucumeris* AG-2-2 on cheese cloth setting up above the potted-plant were kept in a moist chamber. While the inoculated plants were placed in the moist chamber, primary lesion appeared first, followed subsequently by secondary lesions and large-sized irregular lesions.

Germinated basidiospores penetrated the epidermal cell directly, with the formation of an appressorium (Plate 1-4). The invading hypha produced a stroma-like body[10] within the epidermal cell or on the upper layer of the mesophyll (Plate 1-5). After this stage, hyphae grew out radially from that body and caused small, circular primary lesions in 5 or 6 days after the inoculation of spores (Plate 1-6). The stroma-like bodies developed only in basidiospore infection, but not in hyphal infection. It seemed that the bodies were functionally different from the sclerotia[3] which were developed in plant tissues after the disease occurrence, in soil, or on nutrient agar. Stroma-like bodies were also seen on the sugar beet leaves naturally infected. This strongly suggests that basidiospore infection take place in a field.

While continuing to place the diseased plants in the moist chamber, the hyphae growing out from the primary lesions developed and ramified on the leaf surface. The hyphal tips entered the leaf through stomata, causing one or two secondary lesions, 3-5 mm in diameter, around a primary lesion (Plate 1-1). Then, the hyphae growing out from the first secondary lesions entered the leaf through stomata again and formed irregular-shaped lesions with overlapped lesions, finally covering most of the leaf area.

When the diseased leaves with only primary lesions were moved to the dried air glass house, the primary lesions were perforated and fell off, not causing any secondary lesion. A majority of the plants which were moved back to the moist chamber induced secondary lesions.

2) Fructifications and basidiospore dispersal[11]

Perfect stage of *T. cucumeris* on the petiole, the stem and the sheath as well as on

Table 2. Effect of temperatures on fructification of *Thanatephorus cucumeris* AG-2-2 on soil[a]

Temperature (Range) ℃	Duration necessary for basidia formation[b]
15.4 (14.5—15.9)	10
21.5 (19.5—21.8)	4
24.0 (23.3—24.4)	3
28.9 (28.8—28.9)	2

a): Isolate Rh-509 of *T. cucumeris* AG-2-2 was pre-cultured on PDYA containing 0.5% of yeast extracts for 3-4 days and then covered by sterilized soil in a 6 cm petri dish.

b): Days after soil was added.

Table 3. Seasonal variation in number of basidiospores of *Thanatephorus cucumeris* trapped on the 18×18 mm² area of agar plates and development of foliage blight of sugar beets in the field, 1978 (Hokkaido Nat. Agr. Exp. Sta., Sapporo)

Date	Number of basidiospores trapped[a]		Disease severity[b]	
	Height of 20 cm	Height of 60 cm	Inoculated field[c]	Uninoculated field
July 19	25	8	0	0
20	14	1	0	0
21	7	0	0	0
22	1	0	0	0
25	—	—	Trace[d]	0
31	—	2	0.1–0.5	0
Aug. 7	—	—	1.24	0
13	1,965	615	—	—
15	3,106	1,138	—	—
17	4,348	3,033	—	—
18	—	1,708	3.02	0
30	2	0	—	—
Sept. 4	0	0	—	—
8	—	—	3.46	0.61

a): Basidiospores, trapped on the agar plates which were placed horizontally in the air during 17:30 to 8:30 hrs of the next day.
b): Disease severity was rated on a scale of 0 (healthy) — 5 (dead).
c): Some interrow spaces of a sugar beet field was inoculated with anastomosis group AG-2-2 isolate grown on barley grains.
d): First finding of a few circular lesions with small size of 1 mm in diameter.

the soil under the canopy of the leaves of many upland crops and weeds were frequently found in the field during the period the later part of June to the middle of September (Plate 1–3). The isolates which were obtained from the basidiospores were classified in the anastomosis groups AG–2–2, AG–3 or AG–5, and none of them was in the group AG–1. In the sugar beet fields in particular, most fructifications on the surface of petioles bearing root rot lesions and on its surrounding soil under the oldest leaves of the outer whorls belonged to the group AG–2–2. In laboratory experiments, hymenia and basidiospores developed faster on the soil surface in temperatures of 20–29°C than at 15°C (Table 2). In the inoculated field with *T. cucumeris* AG–2–2, fructifications on soil tended to increase with the greater coverage by upland crops.

Relationships between dispersing spores and incidence of foliage blight were investigated. Number of the spores trapped on an agar plate on a slide glass at several heights above the ground surface of sugar beet field indicated that the incidence of foliage blight did not take place on sugar beets until the basidiospores were discharged from the fruit bodies (Table 3). When the secondary lesions developed and enlarged under hot and wet weather conditions, many fruit bodies were found on the healthy surface adjacent to the lesions on the ventral side of the infected leaves. The spore density in the air closely related to increasing distance from the ground surface (Fig. 1), but even at the height of 185 cm in a bean field located 70 m away from the sugar beet field with foliage blight, a number of spores were recorded. In the middle of August when the disease was severe, most spores in the air in the sugar beet and adjacent bean fields were classified in the anastomosis group AG–2–2. Under lower temperature, in early September, no spore dispersal was observed and the incidence of the disease came to cease.

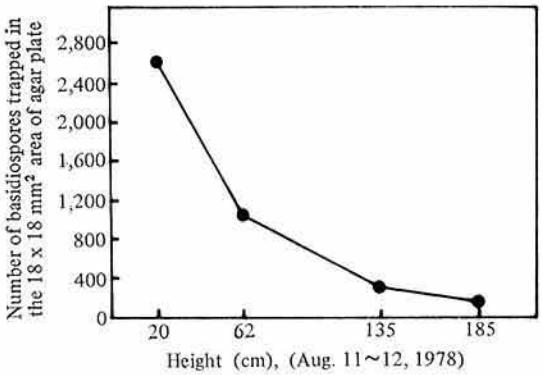


Fig. 1. Vertical distribution of basidiospores of *T. cucumeris* in a sugar beet field (2.3 ha in area), where foliage blight occurred severely under natural conditions
Spores were trapped by an agar plate in the period 17:30 to 8:30 hrs of the next day.

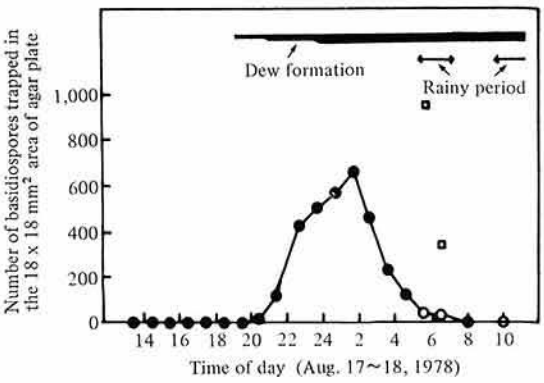


Fig. 2. Diurnal change of basidiospore discharge from basidial mats in the ventral side of a sugar beet leaf in the field
Spores were trapped during a fine or cloudy period (●), during a rainy period (○), under the canopy of an umbrella in a rainy period (□).

Spore dispersal was almost exclusively nocturnal; counts of spores trapped on an agar plate in the air of the sugar beet field started in the evening, increasing rapidly from approximately 9:00 p.m. with maximum in the midnight to dawn (Fig, 2). This time and duration was correlated with dew deposition and relatively high humidity necessary for penetration.

3) Basidiospore germination[9]

The basidiospores of AG-2-2 of *T. cucumeris* sown on a potato sucrose agar began to germinate within five hours at the temperature of 25°C, usually producing one or two germ tubes at each end of the spore, and developed mycelia. The germinated spores on the leaf surface of sugar beets, however, did not all grow up to the mycelia stage. There was no germination in unsterilized soil, while basidiospores were capable of germinating in autoclaved soil.

The optimum temperature for spore germination on an agar medium was 15–32°C. Number of the germinated spores on the sugar beet leaf decreased at the lower temperature, accompanied by the rapid decrease in the percentage of spores infecting leaves at approximately 17°C. Basidiospores germinated only at or above 99% relative humidity, while they lost their germination ability within an hour under direct sun light.

These results indicate that basidospores can survive on a host plant easily by means of infection, though they lose germination ability under unfavorable weather conditions.

4) Survival of pathogen in soil[9]

When blighted leaves of sugar beets were buried in the field soil over a winter, many propagules of *T. cucumeris* on or within diseased tissues persisted until April or May, but soon after this month their viability decreased rapidly. Few propagules, hyphae and sclerotia, survived until the early part of summer and formed fruit bodies on the petioles of the sugar beets and on the surrounding soil as well.

Conclusion

On the basis of the above-mentioned ecological data, the disease cycle could be summarized as presented in Fig. 3. Foliage blight is mainly caused by basidiospores and hyphae of AG-2-2 (root rot type), though AG-1 (web blight type) slightly attacks the leaf with only hyphae. Fruit-bodies, being the source of primary infection, are formed

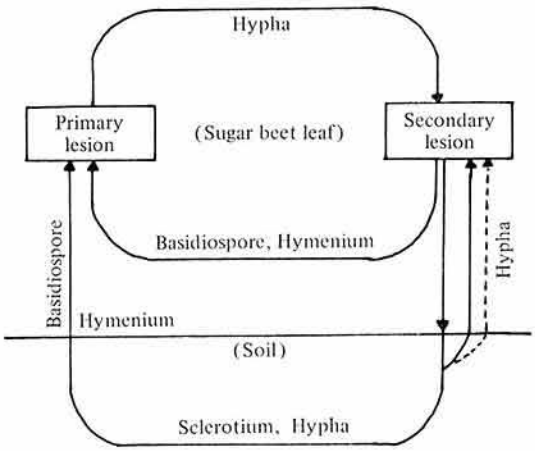


Fig. 3. The disease cylce of foliage blight of sugar beets
A solid line and a dotted line show the disease cycle of AG-2-2 and AG-1, respectively.

on the petioles of sugar beets with root rot disease or on the soil around them, and the primary lesion is caused by the spore dispersing from these fruit-bodies. The hyphae growing out from lesions under the hot and wet weather condition develop the secondary lesions around them. These lesions are enlarged by repeated hyphal infection, resulting in typically blighted leaves. It is rare that the secondary lesions are caused directly by hyphae inhabiting on the soil surface. It is therefore concluded that the disease spreads over a sugar beet field by repeated basidiosporal and hyphal infection. The information of such a disease cycle of *T. cucumeris* provides a basis of controlling root rot of sugar beets. An effective way to reduce foliage blight incidence is first to suppress the basidiospore formation by spraying tolclofos-methyl to the part of crown to control initial infection, and then to repress the disease spreading by spraying mepronil or tolclofos-methyl to crop leaves.

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