

Thanatephorus cucumeris (Rhizoctonia solani AG-2-3) によるダイズ葉腐病の担子胞子感染

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Infection with Basidiospores of *Thanatephorus cucumeris* (AG-2-3 of *Rhizoctonia solani*) and Development of Soybean Foliar Blight Lesions

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Abstract

Soybean leaves artificially inoculated with basidiospores of *Thanatephorus cucumeris* (AG-2-3 of *Rhizoctonia solani*) developed at first, primary lesions, then, secondary lesions and eventually, irregular large-sized lesions. The germ tubes of the germinating basidiospores formed appressoria and their infection pegs penetrated into the epidermal cells. A stroma-like structure was produced within the invaded tissues. Hyphae growing radially from this structure formed circular necrotic spots (≤ 1 mm diam.) which corresponded to the primary lesions. After further incubation in a moist chamber, hyphae from the primary lesions continued to grow on the leaf surface and hyphal tips re-entered the leaves through the stomata, causing secondary lesions with circular to irregularly shaped areas of necrosis around the primary lesions. The large-sized irregular lesions developed from additional hyphal infection through stomata. Many fruiting hymenia of the pathogen were detected on the stems of winter wheat or soybean, especially in fields where soybean was intercropped with winter wheat. The severity of the soybean foliar blight lesions caused by basidiospores increased with the delay in wheat harvest or with the increase of the soybean planting density.

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Key words : soybean, foliar blight, *Thanatephorus cucumeris*, *Rhizoctonia solani* AG-2-3, basidiospore infection, fructification.

INTRODUCTION

Recently, a new farming system for paddy—upland rotation has been implemented in the Tohoku District in the northern part of Japan³⁾. The system consists of three crops growing in the same field for two years, namely, soybean (*Glycine max* (L.) Merr.)—winter wheat (*Triticum aestivum* L.)—intercropped soybean after a rice paddy crop. Since 1991, a new type of foliar disease of soybean caused by *Thanatephorus cucumeris* (Frank) Donk (anamorph = *Rhizoctonia solani* Kühn AG-2-3⁶⁾) has occurred under high relative humidity conditions during the period July through August each year. It appeared especially on soybean intercropped with winter wheat which was planted in fields after rice. Recently, the anastomosis group of *R. solani* AG-2-3 has been proposed as a new subgroup of AG-2. Representatives of this group formed anastomoses with the tester isolates of AG-2-1 but differed by being thiamine auxotrophs⁶⁾.

The symptoms appear as primary lesions consisting of

small, circular necrotic spots, ≤ 1 mm diam., developing under moist conditions into secondary lesions characterized by the presence of circular to irregularly shaped and large-sized areas of necrosis around the primary lesions. The symptoms resemble those of sugarbeet leaf blight and tobacco target spot caused by basidiospores of *T. cucumeris* (*R. solani* AG-2-2 IV⁴⁾ and AG-3^{9,11)}, respectively), rather than those of aerial blight and web blight caused by hyphal infection of *R. solani* AG-1 IA¹⁾ and IB^{1,12)}, respectively. Foliar diseases caused by basidiospore infection have been reported in common bean²⁾ and several crops⁴⁾. However, little is known about the epidemiology of basidiospores causing soybean foliar blight outbreaks.

The present study describes the infection with basidiospores of *T. cucumeris* (AG-2-3 of *R. solani*) and the development of soybean foliar blight lesions.

MATERIALS AND METHODS

Isolates. Isolates R-4 and R-8 of *T. cucumeris* (*R.*

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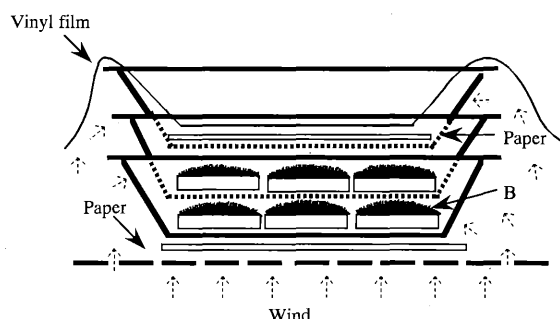


Fig. 1. Formation of basidiospores by *Thanatephorus cucumeris* (AG-2-3 of *Rhizoctonia solani*). After pre-incubation of the fungus in petri dishes, the colonies covered with soil were incubated according to the soil method described by Ogoshi⁸⁾. The plates (B) were placed in piled kitchen baskets on the floor of the growth chamber at 20–25°C. Dotted arrows show wind blowing from the floor of the chamber.

solani AG-2-3) were obtained from soybean leaves collected in a field at Tohoku National Agricultural Experiment Station in Morioka.

Fructification. Formation of hymenia was induced by using the soil method described by Ogoshi⁸⁾. Mycelial agar discs (6 mm diam.) of the isolates were placed in the center of a potato yeast extract dextrose agar plate containing 0.5% glucose and 0.5% yeast extract (pH adjusted to 5.5 by the addition of lactic acid), then incubated at 25°C until mycelial colonies reached the edge of a 9 cm petri dish. The colonies were covered with a 1 cm layer of autoclaved soil (Engei Baido, Kureha Kagaku Co.), from which the excess nutrients were washed out with running tap water for 2 days. The plates were placed in a plastic kitchen basket with 2 mm mesh nets at the bottom and all sides free for aeration (42×36×8 cm in depth, Sekisui Kagaku Co.). Each basket was piled up to two or three-deckers. The bottom of the empty basket on the top was covered with paper and also with a vinyl film to simulate the micrometeorological conditions favorable for sporulation as shown in Fig. 1. A set of two or three-decker baskets was placed on paper (25×40 cm) for avoiding direct wind blowing out from the floor of a growth chamber (mean velocity of the wind in the chamber: less than 0.5 m/sec., Koito Kogyo Co.) at 20–25°C under natural light conditions. The soil moisture in the plates was kept at the water holding capacity level by gently watering the plates daily.

Induction of hymenial formation was artificially carried out in the field, on 17 July in 1992. Isolate R-8 grown on autoclaved barley grains at 25°C for 2 weeks was introduced to the soil (2.5 g/m²) around winter wheat plants (cv. Kitakamikomugi), where soybean plants (cv. Fukunagaha) were sown between the wheat rows, at Tohoku National Agricultural Experiment Station. Basidiospore infection of soybean leaves at the field level was observed from this inoculated field.

Basidiospore inoculation. Soybean seeds (cv. Waseshiroge) were sown in pots, 15 cm in diam., and grown for one month in a glass house. The leaves were inoculated with basidiospores as previously described⁴⁾. Parts of the soil with hymenia layers of *T. cucumeris* (R. *solani* AG-2-3) isolate R-4 were gently picked up using a forceps, and placed on a cheese cloth (Kuremona No. 300) hanging over the potted plants which were put in a cylinder (14 cm diam. by 30 cm height) covered with a vinyl film to maintain a high relative humidity. Discharged basidiospores were dropped on soybean leaves in the moist chamber at 20–25°C for one night. For the control, isolate R-4 grown on autoclaved soybean leaf cuttings (1×1 cm) at 23°C for 7 days was placed in the center of the adaxial surface of the leaves. The inoculated plants were transferred to a moist chamber (1.2×1.2×1.3 m, natural light conditions) with a humidifier⁴⁾ connected to an electric timer at 15 min/hr during daytime and 15 min/3 hr at night. The disease severity was individually rated on a scale of 0 to 5 in which 0 = healthy; 1 = slightly infected; 2 = 1/4 infected; 3 = 1/2 infected; 4 = 3/4 infected and 5 = dead leaf, at 14 days after inoculation with basidiospores and 4 days after inoculation with hyphae.

To determine the optimum temperature for the development of secondary lesions, soybean (cv. Fukunagaha) leaves were inoculated with isolate R-8 grown on a leaf disc (1×1 cm), then placed in a moist chamber at 15, 20, 25 and 28°C at 12 hr intervals of light regime.

To follow the infection process, the diseased leaf discs (1×1 cm) were sampled, fixed and cleared with lactophenol (lactic acid:phenol:water=1:3:10) for 2 or 3 days, then stained with 0.1% cotton blue in lactophenol for microscopic observation.

Anastomosis grouping of basidiospore isolates. In the field surveys carried out from June to September in 1992 and 1993, hymenial development was examined in 25 fields in 7 cities or villages in the Tohoku District. Hymenia were collected from one to four areas/field. Discharged basidiospores were placed on water agar (WA) in 9 cm plates as described by Sinclair¹⁰⁾. A few spores were transferred to potato sucrose agar (adjusted to pH 5 by lactic acid) using a needle under the microscope (×100). Two or three isolates/hymenia were tested for their ability to anastomose with each of the tester isolates from AG-1 to AG-7 and AG-BI. Anastomosis groups were determined as previously described⁶⁾.

Field observation of soybean leaves naturally infected with basidiospores of *T. cucumeris* (R. *solani* AG-2-3). Three crops were grown in the field for two years as follows: soybean—winter wheat—intercropped soybean at Tohoku Natl. Agric. Exp. Stn. The 0.5 ha field was continuously cultivated with soybeans (cv. Fukunagaha) for 2 years (using the standard cultivation practices). After a rice paddy crop in 1989, 2 rows of winter wheat (cv. Koyukikomugi) were planted (Oct. 1, 1991) at a distance of 75 cm. In July, 1992, soybean (cv. Fukunagaha) was planted (41 plants/m² and

17-23 plants/m²) in the 75 cm-interrow space of wheat rows. Wheat was harvested at different times: early harvest (area: 6×140 m, after 1 week), standard harvest (area: 20×140 m, after 2 weeks) and late harvest (area: 6×140 m, after 3 weeks). Control plots consisted of only soybean planted after rice paddy, including soybean (cv. Fukunagaha) cultivated under rotation using standard cultural practices (0.5 ha, 1992) and continuously cultivated for two years (0.5 ha, 1991 and 1992). The plots were arranged in the field adjacent to the soybean-intercropped field. In addition, fields with single soybean cropping and with continuous cropping were selected from standard experimental fields in upland areas of the

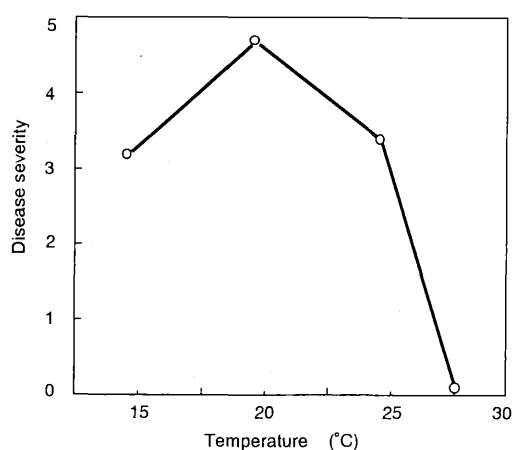


Fig. 2. Effect of temperature on the development of soybean foliar blight caused by *Thanatephorus cucumeris* (AG-2-3 of *R. solani*). Soybean (cv. Fukunagaha) leaves were inoculated with isolate R-8 grown on leaf discs (1×1 cm), then placed in a moist chamber at 12 hr intervals of light regime. Data were recorded after 5 days of incubation.

Station.

RESULTS

Basidiospore infection and development of disease

Germ tubes of germinating basidiospores formed appressoria, and the infection pegs penetrated into the walls of the epidermal cells. The hyphae formed stroma-like structures within the invaded tissues after 4-5 days of inoculation. Subsequently, hyphae grew out radially from these structures (Plate I-2). Primary lesions appeared first as small, necrotic spots (≤ 1 mm diam.) after 6-7 days of inoculation (Plate I-3 and 4). After additional incubation of the diseased plants in a moist chamber, hyphae growing out from the primary lesions penetrated into the leaves through the stomata (Plate

Table 1. Infection of soybean leaves with *Thanatephorus cucumeris* (AG-2-3 of *Rhizoctonia solani*)^{a)}

Inoculum	No. of plants	Blighted leaves (%)	Disease severity ^{b)}
Basidiospores ^{c)}	3	100.0	3.5
Mycelia ^{d)}	3	100.0	2.5
Not inoculated	3	0.0	0.0

a) Soybean plants (cv. Waseshiroge) were grown in a 15 cm diam. pot for one month in a moist chamber kept at 22-25°C.

b) Disease severity data: 0 (healthy) to 5 (completely infected or dead). Data were recorded after 14 days for basidiospore infection, or after 4 days for mycelial inoculation.

c) Basidiospores of isolate R-4 produced on soil were dropped on the leaves in a moist chamber.

d) Leaf disc (1×1 cm) infected with isolate R-4 were inoculated in the center of the leaves.

Table 2. Relationship between hymenial development of *Thanatephorus cucumeris* (AG-2-3 of *Rhizoctonia solani*) and foliar blight severity of soybean in the field^{a)}

Field of soybean	Year with continuous cropping	No. of plants tested	Plant bearing hymenia (%)	Severity ^{b)} of foliar blight
Standard experimental fields converted from paddy fields				
Sole cropping	1	102	0	—
Continuous cropping	2	102	69	++
Intercropping between winter wheat	2	102	49	+++
Standard experimental fields in upland areas				
Sole cropping after wheat	1	102	4	—
Sole cropping after oat	1	102	0	—
Continuous cropping after wheat	5	102	0	—
Field inoculated with AG-2-3 in upland areas				
Inoculated after wheat ^{c)}	1	195	72	+++
Not inoculated after wheat	1	135	0	—

a) Data were collected on 18 August, 1992.

b) Disease severity, rated on a scale of (—): healthy, (+): slight, (++) : middle, (+++) : severe.

c) Barley grains infected with isolate R-8 (AG-2-3) were introduced into the soil (3 July) around winter wheat where soybeans were intersown. Wheat was harvested on 17 July (Tohoku Natl. Agric. Exp. Stn.).

Table 3. Fructification of *Thanatephorus cucumeris* isolates in soybean and winter wheat fields in relation to *Rhizoctonia solani* anastomosis groups^{a)}

Field	Location	Date of sampling	No. of fields	No. of fields with the hymenia of AGs				
				AG				Binucleate <i>Rhizoctonia</i>
				2-3	4	5	Others	
Soybean	Iwate	Aug. 18-Aug. 26	6	5	0	1	0	0
	Fukushima	Sept. 9	1	1	0	0	0	0
	Akita	Aug. 23	1	0	0	1	0	0
Wheat	Aomori	June 29	1	0	1	0	0	0
	Iwate	June 23-July 19	16 ^{b)}	5	0	7	0	7
Total			25	11	1	9	0	7

a) A total 250 basidiospore isolates was collected from 25 fields in 7 cities or villages during the growing season of soybean in 1992 and 1993. Two or 3 representative isolates per area bearing hymenia in a field were used for the determination of the anastomosis groups.

b) Two types of AGs were detected from 3 fields each.

Table 4. Effect of wheat harvest time and soybean planting density on soybean foliar blight development from infection with basidiospores of *Thanatephorus cucumeris* (AG-2-3 of *Rhizoctonia solani*)^{a)}

Harvest time of wheat		Planting density (plants/m ²)	No. of plants tested	Diseased plants (%)	No. of primary lesions/plant
Early	(9 July)	20	20	95.0	7.0
Standard	(16 July)	23	20	100.0	28.7
do	(do)	41	20	100.0	47.7
Late	(23 July)	17	20	100.0	107.9

a) Winter wheat (cv. Koyukikomugi) was planted at a 75 cm row spacing on 1 October in 1991. Soybean (cv. Fukunagaha) was intercropped between the wheat rows on 2 July, 1992. Data were recorded on 8 August, 1992.

I-5) and caused secondary lesions with circular to irregular necrotic spots (3-5 mm diam.). Large-sized irregular lesions developed from additional hyphal infections through the stomata (Plate I-6). Two weeks after inoculation most of the leaves were covered with the enlarged lesions (Table 1). When soybean leaves were inoculated with mycelia, secondary lesions developed directly without any formation of primary lesions with stroma-like structures. Optimum temperature for secondary lesion development was 20°C (Fig. 2).

Barley grains infected with isolate R-8 were introduced into soil around winter wheat plants, in which soybean was intercropped in an upland field at Tohoku Natl. Agric. Exp. Stn. Abundant basidia in hymenia (Plate I-1) were observed on the wheat stems. The discharged basidiospores from these hymenia caused primary lesions with stroma-like structures, followed by secondary lesions on soybean leaves under high relative humidity conditions. On the other hand, neither hymenia nor any foliar blight symptoms could be detected in the non-inoculated field (Table 2). When humid weather conditions persisted, primary and secondary lesions were subsequently formed even after wheat harvest. No basidiospore infection occurred at, or after the soybean pod filling stage.

Fructification on soybean leaves in the field

Hymenia of *T. cucumeris* developed on the stems and soil under the canopy of winter wheat and soybean leaves in upland fields, especially more frequently in the

fields subjected to intertillage or earthing-up. These hymenia developed from isolates belonging to AG-2-3, AG-4, AG-5 of *R. solani*, or to various binucleate *Rhizoctonia* spp. (Table 3). Hymenia were often observed on wheat stems, 0-20 cm above the ground, in late June or early July in fields where soybeans were sown between winter wheat rows. Foliar blight caused by basidiospore infection did not occur until the fruiting bodies were formed (Table 2). When high humidity conditions prevailed in the field, hymenia were occasionally detected on the adaxial surface of soybean leaves.

Cultivation factors affecting foliar blight disease

Wheat harvest time exerted a considerable influence in the development of the primary lesions on soybean leaves after basidiospore infection (Table 4). The numbers of primary lesions/soybean leaf increased with the delay in wheat harvest time. Numbers of primary lesions increased with the increase of the soybean planting density.

DISCUSSION

Soybean foliar blight caused by infection with basidiospores of *T. cucumeris* (AG-2-3 of *R. solani*), affected severely soybean plants in fields where they were intercropped between winter wheat rows⁶⁾. The disease was characterized by symptoms developing from small primary lesions (≤ 1 mm diam.) to secondary large-sized lesions with circular to irregular necrotic

spots. These lesions could be differentiated from those of aerial blight¹²⁾ and web blight^{1,12)} caused by hyphal infection of *R. solani* AG-1 IA and AG-1 IB, respectively, suggesting that the source of infection may differ among the related sources of AGs.

Germinated basidiospore of the isolates of AG-2-3 penetrated into the epidermal cells directly and produced small, circular primary lesions with stroma-like structures within the infected cells. Hyphae of the pathogen grew out from the primary lesions toward healthy leaf surfaces and penetrated into the leaves through the stomata to develop large-sized lesions, when the plants were further incubated under high relative humidity conditions.

Development of primary lesions with stroma-like structures in the field strongly suggests that the basidiospores may be the source of infection. Stroma or stroma-like structures have been previously observed on leaves of tobacco⁹⁾, sugarbeet⁴⁾, *Amaranthus lividus* L. and *Chenopodium album* L. (Naito, unpublished). In all cases, these structures have been detected only after basidiospore infection. Thus, these structures are considered to play an important role in the development of the primary lesions.

Field surveys on fructification of *T. cucumeris* revealed that basidiospores of the AG-2-3 were occasionally produced as an infection source on the stems and on the soil surface under the canopy of winter wheat and soybean before the occurrence of soybean foliar blight outbreaks. The uniform pattern of disease distribution in a field strongly suggests that spore dispersal is playing an important role in the epidemiology of foliar blight. In fact, foliar blight caused by isolates of AG-2-3 did not occur before fructification was evident. However, it remains to be determined why the isolates of the soil-borne fungus AG-2-3 spread and produced many hymenia on wheat stems all over the fields only when soybeans were continuously cultivated for 2 years after rice. Isolates of AG-2-3 have been reported to be highly pathogenic to various leguminous crop plants⁶⁾. They were weakly pathogenic to winter wheat⁶⁾ and not pathogenic at all to rice (Naito, unpublished).

Soybean foliar blight caused by infection with basidiospores of isolates belonging to AG-2-3 was more severe in fields where soybeans were intercropped between winter wheat rows, than in those where only soybean was cultivated. Such phenomena may be explained as follows: frequency of fructification depends more upon the foliar density of the canopy than on the plant species⁴⁾; cultural practices, such as intertillage and earthing up treatments promote hymenial formation by *T. cucumeris* in the field⁴⁾; basidiospores can germinate and penetrate into the host plant leaves only under $\geq 99\%$ relative humidity conditions⁴⁾. The increase in the severity of soybean foliar blight with the delay in wheat harvest or with the increase of soybean planting density may be ascribed to the high relative humidity and its duration under the crop canopy.

Parts of this work have been reported previously in abstracts^{5,7)}.

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和 文 摘 要

内藤繁男・持田秀之・中島 隆・大藤泰雄: *Thanatephorus cucumeris* (*Rhizoctonia solani* AG-2-3) によるダイズ葉腐病の担子胞子感染

Thanatephorus cucumeris (*Rhizoctonia solani* AG-2-3) の担

子胞子を人為的にダイズ葉に接種すると、胞子は付着器を形成して角皮より侵入したのち組織内に stroma 様菌糸塊を形成した。この菌糸塊より菌糸が組織内を放射状に生育し、接種6日あるいは7日後にはじめて直径1 mm またはそれ以下の一次病斑を生じた。さらに湿室に保つと、その病斑から葉面に伸び出した菌糸が気孔より侵入し、周囲に円形～不定形の二次病斑を生じた。その病斑から葉面に現れた菌糸により感染が繰り返されて、

病斑は大形化した。秋播きコムギ立毛時に間作したダイズ畑では、感染源の AG-2-3 の担子胞子は刈取り前のコムギ茎に多数見られた。圃場でも stroma 様菌糸塊を持つ一次病斑とその周囲に不定形の二次病斑とが胞子接種実験と同様な経過で観察された。発病はコムギの刈取り時期が遅れるほど、またダイズの栽植密度が高いほど多い傾向にあった。

Explanation of plate

Plate I

1. Basidia and basidiospores developed on winter wheat stems with intercropped soybean in the field after rice crop. Bar: 40 μm .
2. Stroma-like structure developed within the invaded soybean tissues after artificial inoculation with basidiospores of *Thanatephorus cucumeris* (AG-2-3 of *R. solani*) after 5 days of incubation. Hyphae grew out radially from this structure. Bar: 40 μm .
3. Primary lesion with a stroma-like structure (S) at 6 days after basidiospore inoculation. Bar: 100 μm .
4. Primary lesion (Lp), ≤ 1 mm diam., on a soybean leaf in a field.
5. Hyphal infection through the stomata of a soybean leaf. Bar: 20 μm .
6. Primary lesions (Lp) and secondary lesions (Ls) on a leaf in the field.

Plate I

