Plant Pathology Bulletin 10:88-92, 2001

Inoculum Sources and Host Range of Black Rot of Wasabi Caused by *Phoma wasabiae*

Chaur-Tsuen Lo [1,2] and Kuei-Mai Wang [1]

1. Department of Plant Pathology, Taiwan Agricultural Research Institute, Wu-Feng, Taichung 413, Taiwan, R.O.C.
2. Corresponding author, E-mail: ctlo@wufeng.tari.gov.tw ; Fax: 04-23338162
Accepted for publication: June 15, 2000

ABSTRACT

Lo, C. T., and Wang, K. M. 2001. Inoculum sources and host range of black rot of wasab caused by *Phoma wasabiae*. Plant Pathol. Bull. 10:88-92.

The significance of vegetative propagation as a source of primary inoculum in spread of black rot (or streak disease), caused by *Phoma wasabiae*, was investigated in this study. In tested samples of wasabi plants, 67-90% of the rhizome-tillers and 17-50% of root-plantlets were infected by *P. wasabiae* that spread mainly from infected parent plants to their progeny, tillers and root-plantlets. Results also indicated that six species among 28 tested plants including common weeds also play an important role on initial source. These six species plants grown in/near the wasabi fields were *Alocasia macrorryhiza*, *Bambusa oldhamic*, *Raphanus sativus*, *Boehmeria frutesccus* var. *concolor*, *Rumex japonicus*, and *Sambucus takasagoensis*. Both pycnidia and pycnidiospores of *P. wasabiae* in soils remained infective to the wasabi plant for only a short period of 3 months. Results of this study suggest that wasabi plantings should begin with pathogen-free planting materials to control the disease. In old diseased field, destruction of common weeds and fallow for several months before planting are also essential.

Key words: wasabi, *Phoma wasabiae*, inoculum source, rhizome

Introduction

Black rot (streak disease) of wasabi (*Wasabia japonica* (Miquel) Matsumura) caused by *Phoma wasabiae* Yokogi has been an important disease in Japan and Taiwan [4,5,6,10,13,15,18]. The symptoms on wasabi mainly include streak or black rot on rhizomes, spot on leaves, and blight on petioles [4,10,18]. Particularly, infection at rhizomes of wasabi has become a serious problem in Taiwan in the last 15 years [4,6,10,13]. The disease decreased approximately 30 to 70% of the total production of wasabi rhizome annually, resulting in the poor quality of the rhizome and lower market prices [13,17].

Black rot was first described on wasabi in Japan by Yokogi in 1952, and was first reported in Taiwan in 1986 [4,18]. The disease is widely distributed in Taiwan and several parts of Japan [5,6,13,18]. Wasabi farmers often refer to black rot as "black heart" without distinguishing the causes involved. In 1993, we examined wasabi rhizomes with "black heart" symptoms from 15 fields in four counties in Taiwan. The primary causes included calcium and boron deficiency, and infection by *P. wasabiae*. However, *Phoma wasabiae* was recovered from 90% of the wasabi rhizomes with symptoms of black hearts [6,18].

Farmers in Taiwan are accustomed to planting wasabi via vegetative propagation, using tillers from parent rhizomes or plantlets from fibrous roots of a parent plant. Transmission of black rot of wasabi through vegetative propagation and seed might be the most important way of diseasee spread and the most likely means of introducing the pathogen into previously uninfected areas [1,2,7,14]. However, their significance as primary inoculum has not been documented.

In addition to transmission by propagative materials, pycnidia produced by *P. wasabiae* either on infected plant debris or survival in soils may provide initial inoculum or secondary inoculum as pycnidospores are released from pycnidia exposed to high humidity and disseminated by splashing and windblown rain or sprinkler irrigation [3,8,10,11,12,15,17]. Other crops or weeds can be symptomless hosts [5,12,16] but no studies on host range of *P. wasabiae* have been conducted. The objectives of this study were to determine the primary inoculum sources and host range of the pathogen, which are essential for the developmental of control stratgies.

Materials and Methods

Plant materials

Three cultivars of wasabi, 70-I-1, Tainung No.1, and

Bon-de No.1 (an original local cultivar) [6], were used in this study. Unless indicated otherwise, the vegetative planting materials, including tillers from parent rhizomes and root plantlets from fibrous roots of parent wasabi were provided by wasabi growers in Ali-shan and Ta-pang. The plant materials also included rhizomes and root fragments of parent plants of wasabi. Tillers are the daughter plants that arise from rhizome shoots of parent wasabi. The root plantlets are young plants that arise from fibrous root sprouts of parent wasabi. The plantlets grown from tissue cultures used in this study were provided by the Hsin-Guo Co. (Hsin-Chu, Taiwan). Unless stated otherwise, rhizomes and fibrous root fragments from parent wasabi were grown in sterile sand (10 kg) in boxes (45x30x10 cm) and covered with 1 cm of sterile peat moss (2% Nitrogen, pH 6.0). Each soil box was moistened with 1800 ml of sterile distilled water at the beginning of each experiment. Water was added as needed during the 3-month incubation in a growth chamber at 15-18 ℃ with a 12- h photoperiod to produce the tillers and root plantlets. The tillers and root plantlets were then transplanted to pots with sterile sandy loam and peat moss (1:2, pH 6.0). These plantlets were grown at 15-18℃ with a 12-h photoperiod for 6 months in a growth chamber to obtain adult plants. Fertilizer (N:P:K=15:10:10) was added to the peat-soils as needed.

Media

Potato dextrose agar supplemented with streptomycin sulfate (PDSA) and water agar (WA) were used for isolating *P. wasabiae*. The composition of these media is as follows: PDSA, potato dextrose broth 24 g (Difco Laboratories, Detroit, MI. USA), agar 20 g (Difco), streptomycin sulfate 300 mg (Sigma, Co., MO. USA), distilled water 1000 ml; WA, agar 20g, deionized water 1000 ml.

Survey of disease incidence in wasabi tillers and root plantlets

To understand the relationship between the incidence of wasabi black rot on plantlets from vegetative propagations and their adult (full grown) wasabi plants. Firstly, the disease incidence of rhizomes and fibrous roots, both from parent plants was determined. Secondly, tillers from the infested parental rhizome and root plantlets from parental fibrous roots were examined with PDSA-media to determine their disease incidence of wasabi black rot. Finally, disease incidence of the adult plants grown from tillers and root plantlets were measured. Twenty rhizomes and 100 fibrous roots from parental plants were individually cut into 3- to 5-cm cross-sections with a sterile knife. Five fragments (0.5 x 0.3 cm) taken randomly from each cross-section were placed onto PDSA plates and incubated in a 25℃ incubator for 3 days. The fragments were then examined for infection by the pathogen under a dissecting microscope (30-50X) as were 100 tillers, 100 root plantlets, and 100 adult plants grown for 6 months in growth chamber as described earlier. The disease incidence on these samples was recorded as an initial infection rate. Each experiment had five replications for parent rhizomes and three replications for tillers, root sections, root plantlets, and adult plants. The experiments were repeated twice.

Isolation of *P. wasabiae* and susceptibility of other plant species in/near the wasabi field

To determine the host range of *P. wasabiae*, crops and weeds growing in or near wasabi fields with or without foliar symptoms were collected and planted in sterilized peat soils as described and placed into a growth chamber at 15-20℃ with a 12-h photoperiod provided by cool-white fluorescent lights. The tested plant species are listed in Table 3. In inoculation tests, five plants without symptomatic leaves from each plant species (Table 3) were each misted with 5 ml of spore suspension (10^6 spores/ml) of *P. wasabiae* using a hand-operated trigger-action sprayer, and each immediately was enclosed in a plastic bag. The plastic bag was removed 48 h after inoculation. Control (untreated) plants were treated similarly but received only sterile distilled water. Ten to fourteen days after inoculation at 15-20℃ in a growth chamber, isolation of the pathogen was made from symptomatic infected tissues. The isolated pathogen was identified under a light microscope (400X). The experiments were repeated twice.

Survival of *P. wasabiae* in soil

Fungal propagules surviving in the soil may be an initial inoculum source for the black rot disease caused by *P. wasabiae*. To examine this hypothesis, pycnidia and pycnidiospores were harvested from 3-4 week old PDA plates incubated at 25℃ under constant light. The pycnidia (200 pycnidia/100 g soil) and pycnidiospores (10^6 spores/g soil) were separately and thoroughly mixed in sterilized or natural soils taken from pathogen-infested wasabi fields in Ali-shan. These soils were then put into pots (15 cm in diameter) and incubated for 0, 1, 2, 3, or 4 months in a 15-20℃ growth chamber. Following the incubation period, the soil samples were placed into new pots to which two tissue-cultured plantlets of 70-I-1were planted per pot. Ten pots were used for each treatment. Disease incidence was rated 6 months after transplanting. The experiments were repeated at least twice; Data presented are a representative test.

Data analysis

All data were submitted to analyses of variance, and Duncan's multiple range tests to separate the means using the Statistical Analysis System (SAS Institute Inc., Cary, NC) program.

Results

Examination of disease incidence in tillers, root plantlets, and their mature plants

In the planting materials of wasabi obtained from growers, it was found that 80-90% of rhizomes infected with *P. wasabiae*. Percentages of infection in tillers originated from these rhizomes and adult plants grown from tillers were 67-83% and 82-90%, respectively. The pathogen was isolated from 17-49% of root tissue, 20-50% of root plantlets, and 20-50% of adult plants grown from root plantlets. The cultivar ' Bon-de' has the highest rate of infection in both rhizomes and root tissues as compared to other two cultivars 'Tainug No. 1 and 70-I-1'(Tables 1 and 2).

Susceptibility of other plants in wasabi fields to *P. wasabia*

Most of the 28 plant species tested in or near wasabi fields were not susceptible to *P. wasabiae*. There were only six species of the tested plants showing symptoms on their leaves that were artificially inoculated with spore suspensions of the pathogen (Table 3). They were *Alocasia macrorryhiza* (L.) Schott & Endl., *Bambusa oldhamic* L., *Boehmeria frutesccus* Thunb. var. concolor Nakai., *Raphanus sativus* L, *Rumex japonicus* Hiutt., and *Sambucus takasagoensis* L.

Survival of *P. wasabiae* in soil

Tissue cultured plantlets (70-I-1) were infected by the pathogen when the plantlets were planted in infested soil after being kept for various periods. Both pycnidia and pycnidiospores added to sterile soils remained infective for a period of 2 months. When added to naturally infected soils, they remained infective for 3 months (Table 4). The naturally infested soils lost infectivity beyond 2 months. Symptoms of the black rot disease also appeared in rhizomes of tissue

Table 1. Isolation of *Phoma wasabiae* from parent rhizomes, tillers, and adult plants of the three cultivars wasabi

Cultivar	Pathogen isolation (%) [1]		
	Parent rhizomes	Tillers [2]	Adult plants [2]
70-I-1	85	83	85
Tainung No.1	80	67	82
Bon-de	90	80	90
LSD (p=0.05)	4.3	3.2	4.8

1.Data were taken from 20 samples each of parent rhizomes and 100 samples of tillers and adult plants. Each experiment consisted of five replications for parent rhizomes and three replications for tillers and adult plants. The experiments were repeated twice.

2.Parent rhizomes were incubated in a growth chamber at 15-18℃ with a 12-h photoperiod for 3 months to produce the tillers. The tillers were then transplanted to pots with sterile sandy loam and peat moss (1:2, pH 6.0). These tillers were grown at 15-18℃ with a 12-h photoperiod for 6 months in a growth chamber to obtain adult plants.

Table 2. *Phoma wasabiae* isolation from parent root sections, root plantlets, and adult plants of three cultivars of wasabi

Cultivar	Isolation (%) [1]		
	Root sections	Root plantlets [2]	Adult plants [2]
70-I-1	17	30	30
Tainung No.1	19	20	20
Bon-de	49	50	50
LSD (p=0.05)	6.7	8.9	9.1

1. Data were individually taken from 100 samples each of root sections, root plantlets, and adult plants. Each experiment consisted of three replications and repeated twice.

2. Fibrous root sections of parent wasabi were incubated in a growth chamber at 15-18℃ with a 12-h photoperiod for 3 months to produce root plantlets. Root plantlets were then transplanted to pots with sterile sandy loam and peat moss (1:2, pH 6.0), and grown at 15-18℃ with a 12-h photoperiod for 6 months in a growth chamber to obtain adult plants.

Table 3. In vitro inoculation of plant species found in wasabi field with *Phoma wasabiae*

Plant species	Inoculation with distilled water	Inoculation with [1] *Phoma wasabiae*
Alocasia macrorryhiza	-	+
Alpinia japonica	-	-
Amischotolype chinensis	-	-
Ampelopsis brevipedunculata var. hancei	-	-
Bambusa oldhamic	-	+
Boehmeria frutesccus var. concolor	-	+
Centella asiatica	-	-
Commelina benghalensis	-	-
Crassocephalum crepidioidens	-	-
Cryptomeria japonica	-	-
Digitalis purpurea	-	-
Disporopsis fuscopicta	-	-
Disporum kawakamic	-	-
Drymaria cordata	-	-
Houttuynia cordata	-	-
Languas flabellata	-	-
Marisus cyperinus	-	-
Piper fato-kadsura	-	-
Plantago major	-	-
Poa annua	-	-
Polygonum chinense	-	-
Pteridium aquilinum	-	-
Pyrrosia adnascens	-	-
Raphanus sativus	-	+
Rumex japonicus	-	+
Sambucus takasagoensis	-	+
Setaria palmifolia	-	-
Tricyrtis formosana	-	-
Total		6/28

1. Inoculated plants were grown at 15-20℃ in a growth chamber for 14 days.
Experiments were repeated twice, and data presented are a representative test.

2. "+" represented symptom appreance and "-" = no symptom.

Table 4. Survival of pycnidia and pycnidiospores of Phoma wasabiae in soils as assayed by pathogencity test.

Treatment [3]	% Disease incidence of wasabi rhizome after various treatment duration (months) [2]				
	0	1	2	3	4
Sterilized soil + pycnidiospores	100	75	30	0	0
Sterilized soil + pycnidia	100	30	10	0	0
Natural soil + pycnidiospores	100	20	10	10	0
Natural soil + pycnidia	100	30	10	10	0
Sterilized soil	0	0	0	0	0
Natural soil	30	20	0	0	0

1. The transplanting materials was the tissue-cultured plantlets of wasabi
2. Each treatment was assessed 6 months after transplanting.
3. Treated soils were placed in growth chamber at 15-20℃ for different periods, then planted with tissue-culture plantlets. The experiments were repeated at least twice, and data presented are a representative test.

cultured plantlets in the natural soils without addition of pycnidia or pycnidiospores. However, no disease developed on plantlets after natural soils without an addition of inoculum were placed for 2 months in the same growth chamber (Table 4).

Discussion

Reducing of initial plant infection by pathogens is a reasonable strategy for controlling monocyclic diseases [9]. As with other *Phoma* diseases in general, black rot disease of wasabi is associated with the use of infected asexually propagated plantlets [1,2,7,14]. The results of this study indicated that plantlet transmission of wasabi is the principal source of inoculum for rhizome black rot (or streak disease). Recently, black rot disease in cultivated wasabi fields has more become an important disease of wasabi in Taiwan [10,11]. The mainly causal reason, at least part, is explained that farmers in Taiwan generally use propagative planting methods such as tillers and root plantlets that arise from infected parent plants. Our results in this study demonstrated that the asexually propagative materials of wasabi have had relatively high ratio infection by *P. wasabiae* in Taiwan. There was a close relationship between the extent of progeny plants infection and subsequent incidence of their adult (full grown) wasabi. Therefore, using pathogen-free plantlets will be necessary to control black rot disease of wasabi.

A number of researches indicated that some pathogens could parasitize and survive on several crops and weeds without symptoms [12,16]. Our study indicated that *P. wasabiae* might parasitize at least on six species of tested plants including radish and common weeds in/near wasabi field. The evidence suggests that the pathogen may infect and survive on other plants when the wasabi has been harvested and may serve as initial inoculum for the next growing season. Several researchers reported some weeds in the field had been found capable of harboring pathogen that as well as nonpathogens might invade roots of a variety of plants [16]. In this study, we did not find the information. However, the aspect of invading plant roots merits further study.

In general, airborne pathogens survive longer in plant resides left on the surface than in those buried [2,3]. It is reasonable to believe that fast decay of plant debris below the soil surface facilitates colonization of pycnidia or other structures by other microflora. Our results showed that pycnidia of *P. wasabiae* only survived three months in soils. Consequently, removal or depth-burial of plant debris should reduce the inoculum source and ultimately the severity of black rot in the wasabi field.

Acknowledgements

This work was supported by part of grant (82-AS-2.3-FD-02) from Council of Agriculture, Executive Yuan, Taiwan, ROC.

Reference

1. Adams, M. J. 1980. The role of seed-tuber and stem inoculum in the development of gangrene in potatoes. Ann. Appl. Biol. 96:17-28.
2. Carnegie, S. F., and Cameron, A. M. 1991. The role of soil, seed-potato tuber and haulm in the transmission of *Phoma foveata* from infected seed to daughter tubers. Plant Pathol. 40:352-358.
3. Casela, C. R., and Frederiksen, R. A. 1993. Survival of *Colletotrichum graminicola* sclerotia in sorghum stalk residues. Plant Dis. 77:825-827.
4. Cheng, M. F., and Tung, B. K. 1986. Etiology and fungicide control of black rot of wasabi caused by *Phoma wasabiae*. Journal of National Chia-Yi Institute of Agriculture 13:1-10.
5. Goto, M., and Matsumoto, K. 1986. Causal agents associated with the internal black rot syndrome of Japanese horseradish. Ann. Phytopathol. Soc. Jpn. 52:59-68.
6. Hu, M.F., Chiu S. M., Lo, C. T., Liu, S.Y., and Tu, C. C. 1991. Development of wasabi variety Tainung No. 1. J. Agric. Res. China. 40:13-21.
7. Jacobsen, B.J., and Williams, P. H. 1971. History and control of *Brassica oleracea* seed infection by *Phoma lingam.* Plant Dis. Rep. 55:934-938.
8. Lapwood, D. H., Harris, R. I., and Hide, G. A. 1978. Isolation of *Phoma exigua* var. *foveata* from, and pycnidial formation on, brown watery rots of planted seed potato tubers. Trans. Brit. Mycol. Soc. 71:319-320.
9. Lo, C. T., Nelson, E. B., and Harman, G. E. 1996.

Biological control of turfgrass diseases with a rhizosphere competent strain of *Trichoderma harzianum*. Plant Dis. 80:736-741.
10. Lo, C. T., and Wang, K. M. 2000. Survey of fungal diseases on aboveground parts of wasabi in Taiwan. Plant Pathol. Bull. 9：17-22.
11. Lo, C. T., and Wang, K. M. 2000. Factors affecting pycnidial production and pycnidiospore germination of *Phoma wasabia*, the causal agent of wasabi . Plant Pathol. Bull. 9: 99-106.
12. Lo, C. T., and Sun, S. K. 1987. Distribution and survival of radish yellows pathogen in infested field. Plant Prot. Bull. 29:109-116.
13. Lo, C. T., Wang, K. M., Tu, C. C., and Tsai, W. H. 1990. Studies on streak disease of wasabi. Plant Prot. Bull. 32:345-346 (abstract).
14. Logan, C. 1974. The effect of soil- and tuber-borne inoculum on the incidence of potato gangrene. Ann. Appl. Biol. 78:251-259.
15. Nozu, R., and Yokogi, K. 1932. Streak disease of wasabi. Ann. Phytopathol. Soc. Jpn 2:6.
16. Smith, S.N., and Snyder, W.C. 1974. Persistance of *Fusarium oxysporum* f. sp. *vasinfectum* in fields in the absence of cotton. Phytopathology 65:190-196.
17. Wang, K. M., Lo, C.T., Tu, C. C., and Tsai, W. H. 1992. Effect of temperature and humidity on the development of wasabi streak disease caused by *Phoma wasabiae*. Plant Pathol. Bull. 1:96-103.
18. Yokogi, K. 1952. Studies on the Disease of Wasabi and Their Ccontrol. Res. Bull. Shiamane Pref. Agric. Exp. St. Comm. Issu. 77th Aniv., Found. 3:1-70.

摘　要

羅朝村[1,2]、王貴美[1]. 2000. 山葵黑心病感染源與寄主範圍之探討. 植病會刊 10:88-92. ([1.] 台中縣霧峰鄉農委會農業試驗所；[2.] 聯絡作者，電子郵件:ctlo@wufeng.tari.gov.tw ; 傳真：04-3338162)

本研究主在探討山葵黑心病菌可能危害山葵之主要感染源，以明瞭山葵黑心病菌在田間發生擴展之情形；在測試之山葵植株中，發現山葵黑心病主要感染源來自於農民慣用之分孽苗；依據調結果來自於母株根莖之分孽苗及根苗帶菌率分別為 67-90% 及 17-50%。其他結果亦顯示在測試之二十八植物中，山葵黑心病菌可感染包括山芋、綠竹、山苧麻、蘿蔔、羊蹄、冇骨消等植物，亦即山葵黑心病菌除可感染山葵以外，尚可感染臨近或山葵田中之作物與雜草而成為當期栽培山葵之二次感染源或另一季的初次感染源。另外從測試柄子殼及柄孢子在土壤存活與病害發生之情形，發現含有二者之土壤，需經三個月以上才不會造成根莖黑心病，因此收穫後含有柄子殼或柄孢子之土壤，亦可能是另一季的初次感染源之一。從以上之結果說明減少初次感染源如利用無病苗等將是防治山葵黑心病的必要步驟。

關鍵詞：山葵黑心病菌、山葵、感染源、根莖