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Host specificity and multiplication of eight isolates of *Olpidium brassicae* sensu lato and its related *Olpidium* sp.

Hiroki KOGANEZAWA*, Hiroyoshi INOUE and Takahide SASAYA**

Key words : *Olpidium brassicae* sensu lato, *Olpidium brassicae*, *Olpidium virulentus*, host specificity, strain

Contents

I Introduction

Olpidium brassicae (Wor.) Dang sensu lato (s.l.) is a root-infecting plant parasite and widespread in temperate areas of the world. It was reported to cause a damping-off disease of cabbage, but the disease may be caused by other pathogens[23]. *O. brassicae* s.l. is economically important because *O. brassicae* s.l. transmits several destructive plant viruses[4], such as *Tobacco necrosis virus*, Tobacco stunt virus, *Tulip mild mottle mosaic virus*, *Lettuce big-vein associated virus* (LBVaV) (syn.=Lettuce big-vein virus), *Mirafiori lettuce big-vein virus* (MLBVV) (syn.=Mirafiori

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Department of Agro-environmental Manajement
*Present Kunisada Plant Breeding Station, Kaneko Seeds Co. Ltd.
**Department of Regional Crops Science

lettuce virus) and Tulip streak associated agent[7,9,13,14,15,19].

O. brassicae s.l. is holocarpic and has three developmental stages in its life cycle; zoospore, zoosporangium and resting spore stages. A zoospore with a posterior uniflagellum encysts on epidermal cells of host roots, and cyst protoplasts are injected into the host cytoplasm. Its young thallus develops into either a thin-walled zoosporangium or a thick-walled stellate resting spore. Three to seven days after infection, mature zoosporangia discharge zoospore via exit tubes and repeat its life cycle[18,20,21]. *O. brassicae* s.l. survives for a long period in the absence of the host as resting spores, which also play a critical role for survival of the viruses[3,8].

Previously we demonstrated that the difference of the sexual reproduction existed between crucifer and non-crucifer strains of *O. brassicae* s.l. The single-sporangial isolates of crucifer strains did not develop resting spores alone, however they formed resting spores after mating between isolates with different mating (sexual) types. On the other hand, non-crucifer strains developed resting spores alone without mating. Thus, we suggested that crucifer and non-crucifer strains of *O. brassicae* s.l. are distinct species and we referred to as *O. brassicae* is limited to the fungus whose sexuality is heterothallic and *O. virulentus* (Sahtiyanci) Karling for the fungus whose single-sporangial isolates form resting spores without mating[11]. In this report, we used *O. brassicae* as a heterothallic fungus and *O. virulentus* as a homothallic fungus, furthermore *O. brassicae* s.l. is used to denote both *O. brassicae* and *O. virulentus* for citing earlier reports. One isolate whose resting-spore formation is unknown is referred to *Olpidium* sp..

O. brassicae and *O. virulentus* are intracellular obligate parasites. Most of obligate fungal pathogens have generally very limited host ranges. In contrast, many isolates of *O. brassicae* s.l. infect divergent plants, both monocots and dicots. Barr (1980)[2] listed 50 plant species as host of *O. brassicae* s.l. On the other hand, host specialization of *O. brassicae* s.l. has been known for many years, however, only a few studies on the host specificity of single-sporangial isolates have carried out[6,18,22]. Previously we also reported the host specificity of a single-sporangial isolate WOms-3 from welsh onion[10]. In this report, we expanded the experiment to seven other isolates from diverse hosts to understand the host specificity of *O. brassicae* and *O. virulentus* in Japan. Special emphasis was made on incompatible hosts to create control methods against *O.*

Table 1 Origin of single-sporangial isolates of *Olpidium virulentus*, *O. brassicae* and *Olpidium* sp. used in host-specificity experiments

Isolates		Soil		Bait plants	Propagating plants
		Location	Crop		
O. virulentus	WOms-3	Toyama	welsh onion	oriental melon	oriental melon / cowpea
	TAK-1	Kagawa	tobacco	tobacco	oriental melon / cowpea
	LE-4	Ehime	lettuce	lettuce	oriental melon / cowpea
	WT-1	Wakayama	lettuce	lettuce	oriental melon / cowpea
	F-1	Hiroshima	soybean	oriental melon	oriental melon / cowpea
O. brassicae	CBG-3	Nagano	cabbage	cabbage	cabbage
	YR-2	Shimane	cabbage	cabbage	cabbage / Chinese cabbage
Olpidium sp.	DKN-1	Hiroshima	radish	radish	cabbage / radish

virulentus, which transmits the destructive plant virnses.

II Materials and Methods

1 Isolates of *Olpidium brassicae* s.l. and its related *Olpidium* sp.

Eight isolates used in this experiments was summarized in Table 1. These isolates were trapped by using bait plants from various soils collected at different locations in Japan[11,16].

Five isolates of *O. virulentus* (WOms-3, TAK-1, LE-4, WT-1 and F-1): A single-sporangial isolate WOms-3 obtained from a welsh onion field was kindly provided by Morikawa, Toyama Agricultural Research Center. It has ability to transmit both MLBVV and LBVaV[17]. The host specificity of the isolate was reported previously[10]. TAK-1 was isolated from a tobacco field at Takamatsu, Kagawa Prefecture, and trapped by tobacco. LE-4 was isolated from a lettuce field at Iyo, Ehime Prefecture and trapped by lettuce. The isolate harbored LBVaV which did not induce big-vein symptoms on lettuce. WT-1 was isolated from a lettuce field at Hikigawa, Wakayama Prefecture where lettuce plants showed big-vein symptoms, and trapped by lettuce. The isolate was free from both MLBVV and LBVaV. F-1 was trapped by oriental melon from a soybean field at our institute, Fukuyama, Hiroshima Prefecture.

Two isolates of *O. brassicae* (CBG-3 and YR-2): CBG-3 was isolated from a cabbage field at Fujimi, Nagano Prefecture, and trapped by cabbage with metalaxyl which was applied to prevent damping-off of cabbage seedlings. YR-2 was isolated from a cabbage field infested with clubroot at Hikawa, Shimane Prefecture, and trapped by cabbage. CBG-3 and YR-2 have different sexualities[11].

One isolate of *Olpidium* sp. (DNK-1): DKN-1 was isolated from a radish field at Takano, Hiroshima Prefecture and trapped by a radish. A few stellate resting spores were observed in an initial bulk culture from which single-sporangial isolation was made. However, DKN-1 did not form resting spores by mating with three sister isolates or with nine *O. brassicae* isolates (Koganezawa, unpublished data).

2 Isolation of single sporangium

Single-sporangial isolates were obtained from bulk cultures in oriental melon (*Cucumis melo* var. *makuwa*) cv. Ginsen or cabbage cv. Fujiwase according to Lin et al. (1970)[12]. A single sporangium of *O. virulentus* was transferred to oriental melon, and ones of *O. brassicae* and *Olpidium* sp. were transferred to cabbage. The five isolates of *O. virulentus* were maintained in oriental melon or stored at 4°C in a refrigerator as resting spores in air-dried roots. These isolates were propagated for inoculation either in oriental melon or in cowpea (*Vigna unguiculata* subsp. *unguiculata*) cv. Kurodane-Sanjaku. Because two isolates of *O. brassicae* (CBG-3 and YR-2) and one isolate of *Olpidium* sp. (DKN-1) did not produce resting spores in any hosts tested, they were maintained in living cabbage roots throughout the experiments. Chinese cabbage (*Brassica campestris*) cv. Kukai 70 and radish (*Raphanus sativus*) cv. Oshin were also used for propagating YR-2 and DKN-1, respectively.

3 Culturing of plants

Test plants (Table 2) were grown in plastic strawberry baskets (16×11×6cm) with sea sand[10]. The sand was first passed through a 3mm sieve, washed with tap water (Fukuyama city) to remove dusts and litters, and then sterilized at 121°C for 30min. Seedlings of most plants were germinated on wet filter papers in a Petri dish, and then transplanted into sand. Small seed hosts such as tobacco and celery were sown directly to the sand. The number of seedlings in a basket

Table 2 List of test plants used in host-specificity experiments

Plant species		Cultivar	Japanese name
Scientific name	Common name		
Cucurbitaceae			ウリ科
Cucumis melo var. *makuwa*	oriental melon	Ginsen	マクワウリ
Cucumis sativus	cucumber	Yoshinari	キュウリ
Citrullus lanatus	watermelon	Kohdai	スイカ
Leguminosae			マメ科
Vigna unguiculata	cowpea	Kurodane-sanjaku	ササゲ
Sesbania cannabina	sesbania pea		セスバニア
Glycine max	soybean	Tamahomare	ダイズ
Vigna angularis	adzuki bean	Dainagon	アズキ
Pisum sativum	pea	Kinusaya	エンドウ
Vigna radiata var. *radiata*	mung bean	Green Matpe	リョクトウ
Crotalaria juncea	sunn hemp	Kobutorisoh	クロタラリア
Chenopodiaceae			アカザ科
Beta vulgaris var. *saccharifera*	sugar beet	Sugarmangold	ビート
Spinacia oleracea	spinach	Jiromaru	ホウレンソウ
Chenopodium quinoa			キノア
Compositae			キク科
Chrysanthemum coronatium var. *spatiosum*	garland chrysanthemum	Nakaba Shungiku	シュンギク
Arctium lappa	edible burdock	Akimaki Gobou	ゴボウ
Calendula officinalis	pot marigold		キンセンカ
Tagetes erecta	African marigold		マリーゴールド
Lactuca sativa	lettuce	Cisco	レタス
Umbelliferae			セリ科
Daucus carota	carrot	Honbeni Kintoki	ニンジン
Apium graveolens	celery	Top Seller	セルリー
Cryptotaenia japonica	Japanese hornwort		ミツバ
Solanaceae			ナス科
Lycopersicon esculentum	tomato	Momotarou	トマト
Capsicum annuum	sweet pepper	Ace	ピーマン
Solanum melongena	eggplant	Senryo 2-gou	ナス
Nicotiana tabacum	tobacco	Xanthi nc	タバコ
Cruciferae			アブラナ科
Brassica campestris (rapifera group)	turnip	Kanamachi Kokabu	コカブ
Brassica campestris (pekinensis group)	Chinese cabbage	Kukai 70	ハクサイ
Brassica oleracea (capitata group)	cabbage	Fujiwase	キャベツ
Raphanus sativus (daikon group)	radish	Oshin	ダイコン
Malvaceae			アオイ科
Abelmoschus esculentus	okra	green étude	オクラ
Liliaceae			ユリ科
Allium fistulosum (porrum group)	welsh onion	Kujou Futonegi	ネギ
Allium cepa	onion	Amagashi	タマネギ
Gramineae			イネ科
Oryza sativa	rice	Hinohikari	イネ
Triticum aestivum	wheat	Fukusayaka	コムギ
Festuca arundinacea	tall fescue	Jaguar III	トールフェスク
Avena sativa	oat	Ohtsu one	エンバク

was as follows; 15 for most of plants, and 12 for cucumber and most of legumes. At the time of inoculation the seedlings ranged in age from 5 to 18 days depending on root growth. In earlier experiments all the plants were kept at 20°C in a greenhouse, but in later experiments some plants such as adzuki bean, okra and watermelon were grown at 25–30°C until inoculation. Before inoculation the sand was usually watered with a 1:1,000 or 1:2,000 dilution of Hyponex (N:P:K=8:12:6) or tap water twice a week depending on host species.

4 Inoculation

For inoculation, the zoospore suspension was usually made 2–4 weeks after inoculation, and prior to it, baskets are unwatered for at least four days. The root system was washed free of sand in tap water briefly and immersed in tap water for 10–15 min. The number of zoospores in the suspensions thus obtained varied from 1×10^5 to 7×10^5 per ml in different experiments. The resulting zoospore suspension was pipetted to each seedling at the rate of 3–6 ml per seedling. Prior to inoculation, the sand was nearly saturated with water. If the inoculum did not saturate the sand, tap water was added to give saturation. After inoculation, baskets were placed in plastic trays to prevent contamination, and plants were usually watered with nutrient solution (Hyponex) or tap water at 2, 6, 10, 14 and 17 days after inoculation. Inoculated plants were grown at 20°C in a greenhouse.

5 Counting of zoospores

At 6, 14 and 21 days after inoculation with zoospores, the root systems were removed from the sand and washed briefly by tap water. The number of zoospores was counted after the roots were immersed in a known volume of tap water for 15 min. The volume of water and the number of plants varied depending on the root amount. Usually roots from four to ten plants were immersed in 10 ml water. At 2 and 6 days after inoculation, the growth of the thalli in the inoculated plant roots was observed according to the previous reports[10]. The zoospore were observed using hemacytometers under a differential interference contrast microscope. If a zoospore preparation contained too much zoospore, they were suitably diluted prior to counting. The number of zoospore were divided by the number of plants and expressed as the number per plant.

6 Observation of zoosporangia and resting spores

At 6 and 14 days after inoculation, root pieces were mounted in water and were observed directly under a differential interference contrast microscope for the presence of zoosporangia or resting spores. When roots were too thick to observe, they were pressed between two slide glasses or epidermis were peeled off by a razor blade.

III Results

The host ranges were examined with eight single-sporangial isolates of *O. virulentus, O. brassicae* and *Olpidium* sp. (Table 1). Thirty-six plants belonging to 10 families (Table 2) were inoculated with zoospore suspension. The multiplication of the isolates was assessed by counting number of zoospores released 6, 14 and 21 days after inoculation. The roots of all hosts were also examined for the presence of zoosporangia and resting spores microscopically 6 and 14 days after inoculation to correlate fungal development and zoospore release for each isolate. The results were summarized in Tables 3 and 4.

1 Host compatibility against each isolate

Various aspects of host-parasite relationship were observed, particularly in incompatible

hosts. Based on the observation, hosts were subjectively categorized into five classes; compatible, moderately compatible, slightly incompatible, moderately incompatible and highly incompatible hosts.

In the compatible hosts, each isolate usually produced many mature zoosporangia and released abundant amounts of zoospores, usually more than one million zoospores per plant. In the case of okra, root growth was inhibited due to heavy infection, and a number of zoospores were decreased in second or later generations accordingly.

Moderately compatible hosts were permissive for continuous multiplication. An amount of zoospores were released in the second or later generations. Usually infection rate was lower than in compatible hosts. In some hosts such as wheat, relatively large amount of zoospores were released, however because of the vigorous growth of wheat roots, the density of zoosporangia in the wheat root was low.

In slightly incompatible hosts, a few mature zoosporangia were produced and zoospores were released to some extent in the first or later generations, but few or no zoospores were released from these hosts in the later generations. Generally, the mature zoosporangia were not found two or three weeks after inoculation.

In moderately incompatible hosts, sporangia with a diameter of 10–20 μm or sometimes more were observed, but no zoospores were released. In most cases, thalli seemed to be unable to develop to mature sporangia. In some occasions, normal-appearing resting spores were observed.

In highly incompatible hosts, zoospores cannot invade into root tissues or when infected only primordia with a diameter of 3 to 6 μm (mostly 5 μm) were observed. In some hosts abundant primordia were observed.

Oriental melon, watermelon, cowpea, soybean, eggplant and okra were compatible or moderately compatible hosts for all isolates examined. In addition all the five isolates of *O. virulentus* multiplied in sesbania pea, sugar beet, spinach, *Chenopodium quinoa*, welsh onion and onion. All the isolates did not reproduce or poorly reproduced in celery, sunn hemp, African marigold and oat. Pea, tomato, Japanese hornwort and pot marigold were not suitable hosts for most isolates (Table 4).

All the isolates could penetrate into root cells of most plants tested in almost every combination of host and isolate. Fungal penetration was not observed in only a few cases; TAK-1 in sunn hemp, LE-4 and DKN-1 in African marigold, and F-1 in edible burdock.

2 Host specificity of each isolate

Each isolate reproduced well in many hosts as reported by many investigators, but different isolates exhibited distinct host preferences[6,18,22]. WOms-3 multiplied in many plants. F-1, WT-1, LE-4, TAK-1, and YR-2 had narrower host range in this order. CBG-3 and DKN-1 also showed the narrowest host range. The numbers of compatible and moderately compatible hosts were 26 for WOms-3, 22 for LE-4, 21 for WT-1, 18 for F-1, 16 for TAK-1, 13 for YR-2 and 11 for CBG-3 and DKN-1 among the 36 plants tested.

O. virulentus isolates

The host specificity of WOms-3 was reported previously[10] and is included in this report for comparison [Table 3-(1)]. The zoospore did not release from only four plants, sunn hemp, African marigold, tobacco and cabbage.

TAK-1 did not reproduce well in composite, crucifer and umberiferous plants including lettuce. A large number of zoosporangia were observed in roots of tobacco, eggplant, cowpea and oriental melon, indicating that the isolate can efficiently penetrate into the roots of these plants. However, the isolate did not grow in other solanaceous plants tomato and sweet pepper and

Table 3 Multiplication of single-sporangial isolates of *Olpidium virulentus, O. brassicae*, and *Olpidium* sp.
(1) Isolate WOms-3 from a welsh onion field in Toyama

Plant species[1]	Age at inoculation (day)	Degree of infection[2]	No. of zoospores released per plant[3] ($\times 10^6$)			Compatibility[5]
			6 dpi[4]	14 dpi	21 dpi	
Cucurbitaceae						
oriental melon	7	+++	1.3	5.7	6.8	C
cucumber	7	+++	5.0	6.3	NT	C
watermelon*	7	++	0.3	1.6	1.5	C
Leguminosae						
cowpea	7	+++	10	10	36	C
sesbania pea	7	++	0.4	1.2	NT	C
soybean	7	+++	2.8	6.1	5.2	C
adzuki bean	10	+++	6.9	0.1	NT	C
pea	5	+++	Tr	Tr	NT	SI
mung bean	7	++	Tr	Tr	NT	SI
sunn hemp	7	++	0	0	0	MI
Chenopodiaceae						
sugar beet	7	++	0.2	0.05	NT	MC
spinach	7	++	0.09	0.2	3.1	C
Chenopodium quinoa	7	+++	0.08	0.06	NT	MC
Compositae						
garland chrysanthemum	7	++	Tr	3.6	6.0	C
edible burdock	7	++	0.08	0.3	2.2	C
pot marigold	7	+	0.04	0.04	0.09	MC
African marigold	7	++	0	0	0	MI
lettuce	7	+++	0.02	0.3	1.4	C
Umbelliferae						
carrot	7	++	Tr	1.4	NT	C
celery	10	+	0	Tr	0	SI
Japanese hornwort*	10	+	0	Tr	NT	SI
Solanaceae						
tomato	7	+++	Tr	0.4	1.3	C
sweet pepper	7	+++	0.3	0.5	1.1	C
eggplant	7	+++	0.08	0.7	4.1	C
tobacco	14	(+)	0	0	NT	HI
Cruciferae						
turnip	7	++	0.06	0.07	NT	MC
Chinese cabbage	7	++	0.1	0.2	NT	MC
cabbage	7	(+)	0	0	NT	HI
daikon	5	+	0.3	0	NT	SI
Malvaceae						
okra	10	+++	4.0	3.6	1.6	C
Liliaceae						
welsh onion	10	+++	0.2	0.6	NT	C
onion	10	+++	0.7	1.5	NT	C
Gramineae						
rice	7	+	0.06	0.05	NT	MC
wheat	7	+	Tr	0.06	2.1	MC
tall fescue	7	+	Tr	0.4	2.2	MC
oat	7	+	Tr	Tr	NT	SI

1) See Table 1 for scientific name and cultivar. Asterisks indicate that data were obtained in this study. Others were reported previously[10].
2) Degree of infection is based on the observation at 6 days after inoculation. −: no sporangium, +: more +'s indicate more zoosporangia or resting spores were observed, (+): only primordia were observed.
3) Tr: zoospores were observed, but less than 2 zoospores per 0.1 mm^3 (usually less than $2\text{-}4\times10^4$ per plant), NT: not tested.
4) dpi: days post inoculation.
5) C: compatible, MC: moderately compatible, SI: slightly incompatible, MI: moderately incompatible, HI: highly incompatible. For details, see text.

Table 3 Continued

(2) Isolate TAK-1 from a tobacco field in Kagawa

Plant species[1]	Age at inoculation (day)	Degree of infection[2]	No. of zoospores released per plant[3] ($\times 10^6$)			Compatibility[5]
			6 dpi[4]	14 dpi	21 dpi	
Cucurbitaceae						
oriental melon	7	+++	2.6	1.4	7.0	C
cucumber	7	(+)	0	0	NT	HI
watermelon	7	++	0.3	6.8	NT	C
Leguminosae						
cowpea	7	+++	3.0	1.9	2.4	C
sesbania pea	7	+++	Tr	0.8	1.8	C
soybean	7	+	0.06	0.7	NT	MC
adzuki bean	7	+	Tr	0	NT	SI
pea	5	+	Tr	Tr	NT	SI
mung bean	5	++	0.6	1.8	NT	C
sunn hemp	7	−	0	0	NT	HI
Chenopodiaceae						
sugar beet	7	++	0.04	2.1	NT	C
spinach	7	+++	0.5	1.5	8.4	C
Chenopodium quinoa	7	+++	0.2	0.5	0.9	MC
Compositae						
garland chrysanthemum	7	+	Tr	0	NT	SI
edible burdock	7	+	Tr	0	NT	SI
pot marigold	7	(+)	0	0	NT	HI
African marigold	7	(+)	0	0	NT	HI
lettuce	7	(+)	0	0	NT	HI
Umbelliferae						
carrot	7	+	0	0	NT	MI
celery	10	(+)	0	0	NT	HI
Japanese hornwort	10	+	0	Tr	NT	SI
Solanaceae						
tomato	7	(+)	0	0	NT	HI
sweet pepper	7	(+)	0	0	NT	HI
eggplant	7	+++	0.1	0.7	4.2	C
tobacco	16	+++	1.7	2.3	3.5	C
Cruciferae						
turnip	7	+	Tr	Tr	NT	SI
Chinese cabbage	7	++	0.07	Tr	NT	SI
cabbage	7	++	0.1	Tr	Tr	SI
daikon	5	+	0.1	Tr	NT	SI
Malvaceae						
okra	5	+++	2.8	0.6	2.3	C
Liliaceae						
welsh onion	10	++	0.1	0.1	NT	C
onion	10	+++	0.04	0.5	0.3	C
Gramineae						
rice	7	++	0.05	0.02	NT	MC
wheat	7	+++	1.9	0.8	1.2	MC
tall fescue	7	+	Tr	Tr	NT	SI
oat	7	++	0	0	NT	MI

1) See Table 1 for scientific name and cultivar.
2) -5) SeeTable 3-(1).

Table 3 Continued

(3) Isolate LE-4 from lettuce with *Lettuce big-vein associated virus* in Ehime

Plant species[1)]	Age at inoculation (day)	Degree of infection[2)]	No. of zoospores released per plant[3)] ($\times 10^6$)			Compatibility[5)]
			6 dpi[4)]	14 dpi	21 dpi	
Cucurbitaceae						
oriental melon	7	+++	2.1	8.0	5.9	C
cucumber	7	(+)	0	0	NT	HI
watermelon	7	+++	0.7	7.4	NT	C
Leguminosae						
cowpea	7	+++	1.8	14	5.9	C
sesbania pea	7	+++	1.4	13	NT	C
soybean	7	+++	3.5	2.8	NT	C
adzuki bean	5	+++	2.4	1.5	2.4	C
pea	5	+	Tr	0	NT	SI
mung bean	5	+++	1.0	0.9	1.5	C
sunn hemp	5	+	Tr	0	NT	SI
Chenopodiaceae						
sugar beet	7	+++	0.08	0.3	1.7	C
spinach	7	+++	2.5	4.1	14	C
Chenopodium quinoa	7	+++	0.6	0.9	0.8	MC
Compositae						
garland chrysanthemum	7	+++	Tr	0.8	0.6	MC
edible burdock	7	+	Tr	Tr	NT	SI
pot marigold	7	+	Tr	0	NT	SI
African marigold	7	−	0	0	NT	HI
lettuce	7	+++	0.08	4.1	1.6	C
Umbelliferae						
carrot	7	++	0.1	0.07	NT	MC
celery	10	+	Tr	Tr	NT	SI
Japanese hornwort	10	++	0.03	Tr	NT	SI
Solanaceae						
tomato	7	(+)	0	0	NT	HI
sweet pepper	7	+	Tr	Tr	NT	SI
eggplant	7	+++	0.4	2.4	2.4	C
tobacco	18	+++	0.1	0.3	0.9	MC
Cruciferae						
turnip	7	+++	0.1	0.05	0.1	MC
Chinese cabbage	7	+++	0.2	0.2	0.1	MC
cabbage	7	++	0.03	0.02	NT	MC
daikon	5	+	Tr	Tr	NT	SI
Malvaceae						
okra	7	+++	0.7	0.8	0.7	C
Liliaceae						
welsh onion	10	++	Tr	0.1	0.3	MC
onion	10	++	Tr	1.4	NT	C
Gramineae						
rice	7	+++	Tr	0.1	NT	MC
wheat	5	++	Tr	0.5	2.6	MC
tall fescue	7	+	Tr	0.03	NT	SI
oat	7	(+)	0	0	NT	HI

1) See Table 1 for scientific name and cultivar.

2) -5) SeeTable 3-(1).

Table 3 Continued
(4) Isolate WT-1 from a lettuce field with lettuce big-vein diseuse in Wakayama

Plant species[1]	Age at inoculation (day)	Degree of infection[2]	No. of zoospores released per plant[3] ($\times 10^6$)			Compatibility[5]
			6 dpi[4]	14 dpi	21 dpi	
Cucurbitaceae						
oriental melon	7	+++	2.7	4.3	7.1	C
cucumber	7	+++	3.5	3.3	3.5	C
watermelon	7	+++	3.8	3.4	7.1	C
Leguminosae						
cowpea	7	+++	5.3	12	6.3	C
sesbania pea	7	+++	0.4	1.1	4.5	C
soybean	7	+++	5.9	6.3	3.4	C
adzuki bean	5	+++	4.5	4.5	7.1	C
pea	5	+++	1.1	7.3	2.0	C
mung bean	7	+++	1.7	3.4	1.5	C
sunn hemp	7	++	0	0	NT	MI
Chenopodiaceae						
sugar beet	7	++	0.04	0.06	0.2	MC
spinach	7	+++	1.0	1.1	10	C
Chenopodium quinoa	7	+++	0.07	0.7	2.6	C
Compositae						
garland chrysanthemum	7	+	Tr	0	NT	SI
edible burdock	7	+++	0.5	2.1	1.5	C
pot marigold	7	+	0	Tr	NT	SI
African marigold	7	+	Tr	0	NT	SI
lettuce	7	+++	0.07	0.8	1.6	C
Umbelliferae						
carrot	7	++	0.02	0.1	1.6	C
celery	10	(+)	0	0	NT	HI
Japanese hornwort	10	(+)	0	0	NT	HI
Solanaceae						
tomato	7	+	Tr	Tr	NT	SI
sweet pepper	7	+	Tr	0.4	0.3	MC
eggplant	7	+++	0.4	1.9	5.9	C
tobacco	18	+++	0.02	0.03	NT	MC
Cruciferae						
turnip	7	+	0	0	NT	MI
Chinese cabbage	7	+	Tr	0	NT	SI
cabbage	7	+	Tr	0	NT	SI
daikon	5	+	0	0	NT	MI
Malvaceae						
okra	7	+++	2.7	6.2	3.6	C
Liliaceae						
welsh onion	10	++	0.1	0.2	1.0	C
onion	10	+++	0.05	0.2	0.2	MC
Gramineae						
rice	7	++	Tr	Tr	NT	SI
wheat	5	+	0.2	0.09	0.2	MC
tall fescue	7	+	Tr	0	NT	SI
oat	7	+	0	0	NT	MI

1) See Table 1 for scientific name and cultivar.
2) -5) SeeTable 3-(1).

Table 3 Continued

(5) Isolate F-1 from a soybobean field in our institute at Fukuyama

Plant species[1]	Age at inoculation (day)	Degree of infection[2]	No. of zoospores released per plant[3] ($\times 10^6$)			Compatibility[5]
			6 dpi[4]	14 dpi	21 dpi	
Cucurbitaceae						
oriental melon	7	+++	0.8	2.4	3.5	C
cucumber	7	+++	1.1	0.9	1.1	C
watermelon	7	++	0.08	2.8	0.8	C
Leguminosae						
cowpea	7	+++	2.5	7.9	8.9	C
sesbania pea	7	+++	1.4	1.0	2.8	C
soybean	7	+++	2.4	2.3	0.5	C
adzuki bean	5	+++	7.3	12.5	2.8	C
pea	5	(+)	0	0	NT	HI
mung bean	5	+++	1.4	0.5	3.7	C
sunn hemp	5	+	Tr	Tr	NT	SI
Chenopodiaceae						
sugar beet	7	++	0.02	0.1	NT	MC
spinach	7	+++	0.6	0.7	0.7	MC
Chenopodium quinoa	7	+	Tr	0.1	NT	MC
Compositae						
garland chrysanthemum	7	+	Tr	0	NT	SI
edible burdock	7	−	0	0	NT	HI
pot marigold	7	+	0	0	NT	MI
African marigold	7	+	0	0	NT	MI
lettuce	7	(+)	0	0	NT	HI
Umbelliferae						
carrot	7	+	Tr	0	NT	SI
celery	12	(+)	0	0	NT	HI
Japanese hornwort	10	++	Tr	0.05	NT	MC
Solanaceae						
tomato	7	+	0	0	NT	MI
sweet pepper	7	+++	0.2	1.9	1.5	C
eggplant	7	+++	0.9	1.0	0.5	C
tobacco	18	+	0	Tr	NT	SI
Cruciferae						
turnip	7	+	Tr	Tr	NT	SI
Chinese cabbage	7	++	0.03	Tr	NT	SI
cabbage	7	+	Tr	0	NT	SI
daikon	5	+	Tr	0	NT	SI
Malvaceae						
okra	7	++	0.6	0.6	0.8	C
Liliaceae						
welsh onion	10	+	0.03	0.08	NT	MC
onion	10	++	0.02	0.6	NT	C
Gramineae						
rice	7	++	0.3	0.4	NT	MC
wheat	5	++	0.3	Tr	0	SI
tall fescue	7	+	0	0	NT	MI
oat	7	+	0	0	NT	MI

1) See Table 1 for scientific name and cultivar.
2) -5) SeeTable 3-(1).

Table 3 Continued

(6) Isolate CBG-3 from a cabbage field in Nagano

Plant species[1]	Age at inoculation (day)	Degree of infection[2]	No. of zoospores released per plant[3] ($\times 10^6$)			Compatibility[5]
			6 dpi[4]	14 dpi	21 dpi	
Cucurbitaceae						
oriental melon	7	+	Tr	0.03	0.05	MC
cucumber	7	+	Tr	Tr	0	SI
watermelon	7	++	0.3	0.09	0.05	MC
Leguminosae						
cowpea	7	+++	1.5	2.1	2.3	C
sesbania pea	7	++	0	0	NT	MI
soybean	7	+	0.2	0.1	NT	MC
adzuki bean	5	+	0.2	0.3	NT	MC
pea	5	(+)	0	0	NT	HI
mung bean	7	+	0	0	NT	MI
sunn hemp	7	+	0	0	NT	MI
Chenopodiaceae						
sugar beet	7	+	0	0	NT	MI
spinach	7	+	Tr	0	NT	SI
Chenopodium quinoa	7	+	0	0	NT	MI
Compositae						
garland chrysanthemum	7	+	0	0	NT	MI
edible burdock	7	+	Tr	0	NT	SI
pot marigold	7	(+)	0	0	NT	HI
African marigold	7	(+)	0	0	NT	HI
lettuce	7	+	0	0	NT	MI
Umbelliferae						
carrot	7	+	0	0	NT	MI
celery	10	+	0	0	NT	MI
Japanese hornwort	10	+	0	0	NT	MI
Solanaceae						
tomato	7	(+)	0	0	NT	HI
sweet pepper	7	(+)	0	0	NT	HI
eggplant	7	++	0.08	0.1	1.4	C
tobacco	18	(+)	0	0	NT	HI
Cruciferae						
turnip	7	++	0.9	2.9	2.3	C
Chinese cabbage	7	+++	0.08	0.4	1.2	C
cabbage	7	+++	1.0	1.8	2.4	C
daikon	5	++	0.1	0.8	0.9	MC
Malvaceae						
okra	7	+	0.03	0.03	NT	MC
Liliaceae						
welsh onion	10	+	0	0	NT	MI
onion	10	+	Tr	0	NT	SI
Gramineae						
rice	7	(+)	0	0	NT	HI
wheat	5	+	0.02	Tr	0	SI
tall fescue	7	(+)	0	0	NT	HI
oat	5	(+)	0	0	NT	HI

1) See Table 1 for scientific name and cultivar.
2) -5) SeeTable 3-(1).

Table 3 Continued

(7) Isolate YR-2 from a cabbage field in Shimane

Plant species[1]	Age at inoculation (day)	Degree of infection[2]	No. of zoospores released per plant[3] ($\times 10^6$)			Compatibility[5]
			6 dpi[4]	14 dpi	21 dpi	
Cucurbitaceae						
oriental melon	7	++	0.2	0.05	0.03	MC
cucumber	7	+	0.06	0	Tr	SI
watermelon	7	++	0.1	0.06	0.05	MC
Leguminosae						
cowpea	7	+	0.5	2.2	0.09	MC
sesbania pea	7	+	0	0	NT	MI
soybean	5	++	0.2	0.2	NT	MC
adzuki bean	5	+++	0.8	1.6	1.4	C
pea	5	+	0	0	NT	MI
mung bean	5	+	0.07	0.3	0.1	MC
sunn hemp	5	+	0	0	NT	MI
Chenopodiaceae						
sugar beet	7	+	0	0	NT	MI
spinach	7	++	0	0	NT	MI
Chenopodium quinoa	7	+	0	0	NT	MI
Compositae						
garland chrysanthemum	7	(+)	0	0	NT	HI
edible burdock	7	+	0	0	NT	MI
pot marigold	7	+	0	0	NT	MI
African marigold	7	(+)	0	0	NT	HI
lettuce	7	++	Tr	0	NT	SI
Umbelliferae						
carrot	7	(+)	0	0	NT	HI
celery	12	+	0	0	NT	MI
Japanese hornwort	10	(+)	0	0	NT	HI
Solanaceae						
tomato	7	(+)	0	0	NT	HI
sweet pepper	7	(+)	0	0	NT	HI
eggplant	7	+	0.3	0.9	2.9	C
tobacco	18	(+)	0	0	NT	HI
Cruciferae						
turnip	7	+++	0.7	7.5	13	C
Chinese cabbage	7	+++	3.7	5.4	14	C
cabbage	7	+++	0.2	2.2	3.4	C
daikon	5	++	0.06	2.4	5.7	C
Malvaceae						
okra	7	++	0.06	0.04	Tr	MC
Liliaceae						
welsh onion	10	(+)	0	0	NT	HI
onion	10	+	Tr	0.1	0.08	MC
Gramineae						
rice	7	(+)	0	0	NT	HI
wheat	5	+	Tr	0	NT	SI
tall fescue	7	+	Tr	0	NT	SI
oat	5	(+)	0	0	NT	HI

1) See Table 1 for scientific name and cultivar.
2) -5) SeeTable 3-(1).

Table 3 Continued

(8) Isolate DKN-1 from a radish field in Hiroshima

Plant species[1]	Age at inoculation (day)	Degree of infection[2]	No. of zoospores released per plant[3] (×10⁶)			Compatibility[5]
			6 dpi[4]	14 dpi	21 dpi	
Cucurbitaceae						
oriental melon	7	++	0.2	0.2	0.2	MC
cucumber	7	+	0.1	Tr	Tr	SI
watermelon	7	+++	0.2	0.08	Tr	MC
Leguminosae						
cowpea	7	++	0.3	0.3	0.2	MC
sesbania pea	7	+	Tr	Tr	NT	SI
soybean	7	++	0.2	0.07	NT	MC
adzuki bean	5	+	0.3	0.6	NT	MC
pea	5	+	0	0	NT	MI
mung bean	5	+	Tr	Tr	NT	SI
sunn hemp	5	+	Tr	Tr	NT	SI
Chenopodiaceae						
sugar beet	7	+	0	0	NT	MI
spinach	7	(+)	0	0	NT	HI
Chenopodium quinoa	7	+	Tr	0	NT	SI
Compositae						
garland chrysanthemum	7	+	0	0	NT	MI
edible burdock	7	(+)	0	0	NT	HI
pot marigold	7	(+)	0	0	NT	HI
African marigold	7	−	0	0	NT	HI
lettuce	7	(+)	0	0	NT	HI
Umbelliferae						
carrot	7	+	0	0	NT	MI
celery	12	(+)	0	0	NT	HI
Japanese hornwort	10	(+)	0	0	NT	HI
Solanaceae						
tomato	7	(+)	0	0	NT	HI
sweet pepper	7	+	Tr	0	NT	SI
eggplant	7	+	Tr	0.1	NT	MC
tobacco	18	(+)	0	0	NT	HI
Cruciferae						
turnip	7	+++	1.7	3.7	1.4	C
Chinese cabbage	7	+++	2.2	4.7	2.1	C
cabbage	7	+++	1.6	3.8	8.6	C
daikon	5	+++	3.3	10	35	C
Malvaceae						
okra	5	+	0.06	0.7	Tr	MC
Liliaceae						
welsh onion	10	+	Tr	0	NT	SI
onion	10	+	Tr	0	NT	SI
Gramineae						
rice	7	+	0	Tr	NT	SI
wheat	5	+	Tr	0	NT	SI
tall fescue	7	(+)	0	0	NT	HI
oat	7	(+)	0	0	NT	HI

1) See Table 1 for scientific name and cultivar.
2) -5) SeeTable 3-(1).

Table 4 Summary of host specificity of *Olpidium* isolates

Plant species[1)]	*O. virulentus*					*O. brassicae*		*Olpidium* sp.
	WOms-3	TAK-1	LE-4	WT-1	F-1	CBG-3	YR-2	DKn-1
Cucurbitaceae								
oriental melon	C[2)]	C	C	C	C	MC	MC	MC
cucumber	C	HI	HI	C	C	SI	SI	SI
watermelon	C	C	C	C	C	MC	MC	MC
Leguminosae								
cowpea	C	C	C	C	C	C	MC	MC
sesbania pea	C	C	C	C	C	MI	MI	SI
soybean	C	MC	C	C	C	MC	MC	MC
adzuki bean	C	SI	C	C	C	MC	C	MC
pea	SI	SI	SI	C	HI	HI	MI	MI
mung bean	SI	C	C	C	C	MI	MC	SI
sunn hemp	MI	HI	SI	MI	SI	MI	MI	SI
Chenopodiaceae								
sugar beet	MC	C	C	MC	MC	MI	MI	MI
spinach	C	C	C	C	MC	SI	MI	HI
Chenopodium quinoa	MC	MC	MC	C	MC	MI	MI	SI
Compositae								
garland chrysanthemum	C	SI	MC	SI	SI	MI	HI	MI
edible burdock	C	SI	SI	C	HI	SI	MI	HI
pot marigold	MC	HI	SI	SI	MI	HI	MI	HI
African marigold	MI	HI	HI	SI	MI	HI	HI	HI
lettuce	C	HI	C	C	HI	MI	SI	HI
Umbelliferae								
carrot	C	MI	MC	C	SI	MI	HI	MI
celery	SI	HI	SI	HI	HI	MI	MI	HI
Japanese hornwort	SI	SI	SI	HI	MC	MI	HI	HI
Solanaceae								
tomato	C	HI	HI	SI	MI	HI	HI	HI
sweet pepper	C	HI	SI	MC	C	HI	HI	SI
eggplant	C	C	C	C	C	C	C	MC
tobacco	HI	C	MC	MC	SI	HI	HI	HI
Cruciferae								
turnip	MC	SI	MC	MI	SI	C	C	C
Chinese cabbage	MC	SI	MC	SI	SI	C	C	C
cabbage	HI	SI	MC	SI	SI	C	C	C
daikon	SI	SI	SI	MI	SI	MC	C	C
Malvaceae								
okra	C	C	C	C	C	MC	MC	MC
Liliaceae								
welsh onion	C	C	MC	C	MC	MI	HI	SI
onion	C	C	C	MC	C	SI	MC	SI
Gramineae								
rice	MC	MC	MC	SI	MC	HI	HI	SI
wheat	MC	MC	MC	MC	SI	SI	SI	SI
tall fescue	MC	SI	SI	SI	MI	HI	SI	HI
oat	SI	MI	HI	MI	MI	HI	HI	HI

1) See Table 1 for scientific name and cultivar.

2) C: compatible, MC: moderately compatible, SI: slightly incompatible, MI: moderately incompatible, HI: highly incompatible. For details, see text.

in a cucurbit plants cucumber. The isolate did not reproduce well in adzuki bean in which other isolates reproduced well. No infection was observed in sunn hemp [Table 3-(2)].

LE-4 reproduced well in two cucurbits, three legumes, three chenopodiaceous plants, lettuce and eggplant (compatible host). Zoospores were not released from cucumber, African marigold, tomato and oat (highly and moderately incompatible host) [Table 3-(3)].

WT-1 had host specificity similar to that of LE-4. The WT-1 reproduced well in cucumber, pea and edible burdock in which LE-4 could not reproduce or poorly reproduced. Another difference between WT-1 and LE-4 was that WT-1 did not reproduce in crucifer plants. WT-1 infected mainly root hair and root hair cells of pea in which other isolates did not multiply [Table 3-(4)].

F-1 reproduced in many hosts with adzuki bean as the most compatible hosts. In adzuki bean, F-1 grew rapidly and released zoospores two days after inoculation. It did not reproduce well in composite and crucifer plants similarly to TAK-1 [Table 3-(5)].

***O. brassicae* and its related isolates**

CBG-3 reproduced well in all four crucifer plants. It also multiplied in seven non-crucifer plants, among which cowpea and eggplant were compatible hosts. Polymorphic zoosporangia similar to those in crucifer plants were observed in roots of oriental melon, watermelon, onion and eggplant, but the degree of infection in these plants was lower than that of crucifer plants. There were a small number of mature spherical zoosporangia and a lot of small primordia in the roots of okra. In cucumber, soybean, spinach, adzuki bean, edible burdock and wheat a small number of spherical zoosporangia were observed. No zoospores were released from 18 plants in which the development of thalli was arrested prior to maturation of the sporangia [Table 3-(6)].

The host specificity of YR-2 was similar to that of CBG-3. However, YR-2 released more zoospores from several plants than CBG-3 and multiplied in mung bean and onion which were incompatible or slightly incompatible hosts for CBG-3. YR-2 reproduced more in turnip and Chinese cabbage than in cabbage, though it was isolated from a cabbage field [Table 3-(7)].

The host specificity of DKN-1 was very similar to that of CBG-3. 4 major difference between DKN-1 and CBG-3 was that DKN-1 reproduced well in radish [Table 3-(8)].

IV Discussion

In this report, we examined the host specificity of eight isolates of *Olpidium* species resembling *O. brassicae* s.l. by counting the number of zoospores. The counting was performed at 6, 14 and 21 days after inoculation. Since usually *O. brassicae* s.l. releases a large number of zoospores from day 3 through day 6 after inoculation under the suitable condition[6], most of the zoospores at 6 days after inoculation were considered to represent nearly the total zoospore production from the first generation of vegetative reproduction and at 14 days came from the second to fourth generations. In a few host-isolate combinations such as F-1 in adzuki bean and DKN-1 in cabbage, since the zoospores were released at 2 days after inoculation, the zoospores at 6 days might come from their second generation.

The host-range of single-sporangial isolates of *O. brassicae* s.l. was only reported by Sahtiyanci (1962)[18], Temmink et al. (1970)[22], and Campbell and Sim (1994)[6]. *O. brassicae* s.l. isolated from lettuce and tomato was regarded as plurivorous with little specificity[18,22]. On the other hand crucifer isolates and an oat isolate had narrower host ranges[18,22]. Campbell and Sim (1994)[6] reported the host specificity of four isolates of *O.*

brassicae s.l. using 9 plant species. A lettuce isolate reproduced in many hosts. An isolate from a squash field was plurivorous with red clover as the most compatible host. Two isolates limited to cucurbits plants. Melon and watermelon were common hosts for all four isolates. In our experiments oriental melon and watermelon were common hosts for all isolates. Temmink et al. (1970)[22] reported that cowpea and sugar beet were suitable hosts for reproduction of all their four isolates including the mustard isolate. Campbell and Lin (1976)[5] used cowpea as a common host for their four isolates. Our results also showed that cowpea was compatible or moderately compatible hosts for all isolates. Probably cowpea, melon including oriental melon and watermelon are common hosts of *O. virulentus* and *O. brassicae*. In addition all our isolates multiplied in soybean, okra and eggplant.

Since our previous report showed crucifer and non-crucifer strains of *O. brassicae* s.l. are distinct species[11], in this report we refer to the fungus whose single-sporangial isolates form resting spores without mating as *O. virulentus*. *O. brassicae* is limited to those who form resting spores after mating as suggested by Sahtiyanci (1962)[18]. The isolate DKN-1 is referred to *Olpidium* sp., because it did not form resting spores even by mating with sister isolates and *O. brassicae* isolates. The morphology of zoosporangia and zoospores was same as that of *O. brassicae* (Koganezawa, unpublished data), moreover, the host range of DKN-1 was very similar to that of *O. brassicae* isolates CBG-3 and YR-2. Probably DKN-1 is *O. brassicae,* but it may belong to another mating group differed from CBG-3 and YR-2. Our isolates of *O. brassicae* and *Olpidium* sp. showed the similar host specificity, but the different degree of their multiplication in several host plants. DKN-1 multiplied well in radish, while YR-2 multiplied well in turnip and Chinese cabbage. Compatible hosts for the three isolates were mainly limited to crucifer plants. The three isolates also infected several other non-crucifer plants as reported by Sahtiyanci (1962)[18] and Temmink et al. (1970)[22], but their multiplication was usually lower in non-crucifer plants than in crucifer plants except a few host-isolate combinations. Sahtiyanci (1962)[18] reported that *P. brassicae* (=*Olpidium brassicae*) infected eggplant, spinach and beet. According to Temmink et al. (1970)[22], their mustard isolate infected cowpea, spinach, beet and tobacco, but our isolates did not multiply in spinach, beet and tobacco, indicating that our Japanese isolates are different from the foreign isolteas in host specificity.

In contrast to our Japanese isolates of *O. brassicae*, five isolates of *O. virulentus* exhibited a great variability in host specificity as reported by other investigators[6,22]. Factors determining host specificity is currently unknown. Since primordia or aborted thalli were observed in roots of most incompatible hosts or even in moderately compatible hosts, host specificity is probably determined during development to sporangia after cyst protoplasts enter the host cell as suggested by Campbell and Sim[6].

Lettuce big-vein and tulip mild mottle mosaic diseases are major serious virus diseases transmitted by *O. virulentus* in Japan[1,16]. In lettuce and tulip fields, a rotation cropping with paddy rice during summer is a common practice in the diseases endemic areas. Four isolates of *O. virulentus* multiplied in rice to some extent. Although isolate WT-1 released few zoospores from rice roots, it formed relatively abundant resting spores in the roots (data not shown). Therefore the paddy rice plants may play an important role as a reservoir of viruses and vectors.

Interestingly African marigold, sunn hemp and oat are either slightly incompatible, incompatible or highly incompatible hosts for all isolates examined. The cultivars of theses plants used in

the experiments are known as nematode suppressive crops. The facts may pave the way for the development of method to control of *Olpidium*-transmissible virus diseases such as lettuce big-vein by using nematode suppressive crops as a cleaning crop. Another possible and feasible method to control *Olpidium*-transmissible virus diseases is to avoid growing crops susceptible to *Olpidium* species. All the isolates of *O. virulentus* did not multiply and reproduce zoospores well in radish which may be suitable as an alternative crop in big-vein prone fields. Pea and cabbage would be also effective, if *O. virulentus* in the fields does not grow in these plants.

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Summary

The host specificity of eight single-sporangial isolates of *Olpidium* species resembling *O. brassicae* sensu lato were examined by inoculating 36 plant species of 10 families in sea-sand culture with zoospore suspension. The multiplication of the isolates was assessed by counting number of zoospores and by observation of zoosporangia and resting spores in host roots. Among the isolates of *O. virulentus*, Woms-3 had the widest host range, followed by LE-4, WT-1, F-1, and TAK-1. These isolates showed distinct host specificities. The isolates of *O. brassicae*, CBG-3 and YR-2 as well as *Olpidium* sp. DKN-1 had relatively a narrower host range similar to each other. Oriental melon, watermelon, cowpea, soybean, eggplant and okra were compatible or moderately compatible host for all isolates. All isolates had high ability to penetrate into root cells of different plants. The penetration was not observed in only four cases. All the isolates, if infected, did not grow or poorly reproduced zoospores in celery, and in nematode suppressive crops sunn hemp, African marigold and oat.

Olpidium brassicae sensu lato および類似菌の8分離株の宿主特異性並びに増殖

小金澤碩城*・井上博喜・笹谷孝英**

摘　　要

海砂で栽培した10科36種の供試植物に *Olpidium brassicae* sensu lato およびその類似菌の単遊走子嚢分離8株を接種し，一定期間後に放出される遊走子数を計測し，かつ根内の遊走子嚢と休眠胞子を観察することにより，寄生性を調査した．*O. virulentus* の分離株 WOms-3 は最も広い寄主範囲を示した．ついで LE-4，WT-1，TAK-1，F-1分離株の順であった．また，それぞれの分離株は異なる宿主特異性を有していた．これに対し，*O. brassicae* の分離株 CBG-3，YR-2と *Olpidium* sp. の分離株 DKN-1の寄生範囲は比較的狭く，かつ類似していた．いずれの分離株も多くの植物の根に侵入可能で，感染が認められなかったのは4例のみであった．いずれの分離株もマクワウリ，スイカ，ササゲ，ダイズ，ナスとオクラでは増殖可能であった．いずれの分離株もセルリーと線虫抑止作物のクロタラリア，マリーゴールド，エンバクでは増殖しないかあるいは増殖量は少なかった．

地域基盤研究部
*現カネコ種苗株式会社　くにさだ農場
**特産作物部