# Identification of Bremia lactucae races on Lactuca sativa in Kagawa Prefecture and assessment of host range in wild Asteraceae in Japan

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# Abstract

Two *Bremia lactucae* isolates were obtained from infected lettuce (*Lactuca sativa*) plants cultivated in fields in Kagawa Prefecture, Japan. The two isolates exhibited distinct reaction phenotypes compared to those of races previously characterized by the International Bremia Evaluation Board and detected in Hyogo Prefecture, suggesting the presence of previously unreported races in Japan. Commercial lettuce cultivars with resistance genes showed minimal sporulation, confirming their effectiveness for disease control. Cross-inoculation experiments with *B. lactucae* on wild *Lactuca serriola* collected from three regions reveled sporulation only on plants from Hiroshima Prefecture. Furthermore, *L. sativa* cotyledons inoculated with conidia from *L. serriola* also developed sporulation, providing evidence of cross-infection of *B. lactucae* between *L. sativa* and *L. serriola* in Japan. The detection of new *B. lactucae* races, as well as the regional susceptibility of *L. serriola* to *B. lactucae*, may be influenced by factors such as weed management and crop rotation practices, including paddy rice cultivation in Japan, compared to field crops in other countries.


# Introduction

Lettuce (*Lactuca sativa*) is one of the most widely cultivated vegetables worldwide. In Japan, annual production exceeds 550,000 tons (E-stat 2023). *Bremia lactucae*, the causal agent of lettuce downy mildew, is a major pathogen in both field-grown and greenhouse lettuce globally. *Bremia lactucae* consists of numerous physiological races, which are classified based on their virulence patterns against differential lettuce genotypes carrying known dominant resistance (*Dm*) genes. To date, 51 *Dm* genes have been reported (Parra et al. 2016). The International Bremia Evaluation Board (IBEB), a collaborative effort among lettuce breeding companies in several countries, works to identify new *B. lactucae* races that pose significant threats to the lettuce industry and to standardized race nomenclature worldwide (IBEB 2025).

While lettuce is the primary host, *B. lactucae* can infect many Asteraceae species, with over 230 host species reported globally (Morgan 1981). In Brazil, for example, it has been found on *Cynara scolymus* (Mendes et al. 1998) and on *Sonchus asper* and *Sonchus oleraceus* (Vieira and Barreto 2006). In Europe, 17 *Lactuca* species are known to be susceptible, though only seven are considered natural hosts (Lebeda et al. 2001, 2002). Among these, *Lactuca serriola* is the most common wild host in countries such as the Netherlands (Hooftman et al. 2006) and the Czech Republic (Petrželová and Lebeda 2004; Lebeda et al. 2007, 2008). Natural populations of *L. serriola* in Europe exhibit high variation in race-specific resistance to *B. lactucae*, with diverse resistance phenotypes across regions (Lebeda et al. 2008; Petrželová and Lebeda 2011). In Japan, Nishiguchi and Futai (2010) described several races of *B. lactucae* in Hyogo Prefecture, but detailed studies of wild hosts have not been conducted.

In this study, we investigated *B. lactucae* races affecting *L. sativa* cultivated in Kagawa Prefecture, Japan, during 2016 and 2017, and isolated two new races. We also assessed the resistance of commercial lettuce cultivars to these races and examined the host range in wild Asteraceae species.

These findings provide new insights into the occurrence and distribution of *B. lactucae* on *L. serriola* in Japan.

## Materials and methods

# Isolates and maintenance of host plants

Two isolates of *B. lactucae* were collected from infected lettuce (*Lactuca sativa* cv. Ciscoviva) plants grown in fields in Kagawa Prefecture in 2016 and 2017. The isolates were designated KKB001 (2016) and KKB002 (2017), with each isolate obtained from a single infected leaf.

The isolates were maintained and propagated on seedlings of *L. sativa* cv. Ciscoviva. Seeds were sown in a commercial potting soil (Yosaku N-150; Jcam Agri Co., Ltd., Tokyo, Japan) in15-cm terracotta pots. The pots were placed in a growth chamber (Biotron LH-220S, Nippon Medical & Chemical Instruments Co., Ltd., Osaka, Japan) set at 20 ℃ under a 16:8-h L:D photoperiod and watered regularly to maintain optimal moisture levels.

Fifteen-day-old seedlings (with fully expanded cotyledons) were inoculated by spraying with a conidial was were placed in a plastic bag containing 30 mL of tap water and shaken manually. After shaking, the leaf was removed and the resulting suspension was adjusted to a concentration of $1.0 \times 10^5$ conidia/mL. This suspension was then sprayed evenly onto the seedlings.

Following inoculation, the seedlings were incubated overnight at 15°C and 100% relative humidity in darkness for 8 h to allow infection. Thereafter, they were transferred to under a 16:8-h photoperiod and natural humidity conditions at 10°C for 20 days. During this period, the seedlings were sprayed with tap water and incubated overnight at 15°C and 100% relative humidity. Unfortunately, the KKB001 isolate was broken during the above maintenance, so the used isolates vary by test.

## Morphology and nucleotide sequence analyses

The isolates were morphologically identified as *B. lactucae* by microscopic examination of conidiophores and conidia using a BX51 microscope (Olympus Corporation, Tokyo, Japan).

For molecular identification, genomic DNA was extracted from diseased leaves using a DNeasy Plant Mini Kit (Qiagen, Germantown, MD, USA). Polymerase chain reaction (PCR) amplification was performed in 20-μL reaction mixtures containing 10 μL of polymerase mix (AmpliTaq Gold 360 Master Mix; Thermo Fisher Scientific, Waltham, MA, USA), 0.2 μM of each primer, and 25 ng/μL of template DNA.

Two DNA gene regions were amplified: (1) the large subunit ribosomal RNA (LSU rDNA) region using primers LR0R (Moncalvo et al. 1995) and LR6-O (Riethmuller et al. 2002), and (2) the cytochrome c oxidase subunit 2 (cox2) region using Cox2-F and Cox2-R (Hudspeth et al. 2000). PCR was performed using a Takara PCR Thermal Cycler Dice Touch (Takara Bio Inc., Kusatsu, Japan) under the following

cycling conditions: initial polymerase activation at 95°C for 9 min; 36 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 40 s, and extension at 72°C for 1 min; followed by final extension at 72°C for 5 min.

The amplified PCR products were purified using a MonoFas DNA Purification Kit I (Animos Inc., Kawaguchi, Japan) and subsequently sequenced by a commercial sequencing service (Eurofins Scientific SE, Luxembourg, Luxembourg). Sequence analyses were conducted using MEGA v. 10.0 (Kumar et al. 2018).

### Determination of B. lactucae races

Race determination was conducted following the methods of Nishiguchi and Futai (2010) with minor modifications. Each of the two isolates (KKB001 and KKB002) was inoculated onto lettuce plants, with two replicate tests performed for each isolate.

The differential set of *L. sativa* seeds used for race testing was kindly provided by Professor Richard W. Michelmore (University of California, Davis, CA, USA). Seeds of 25 differential cultivars were sown individually in Petri dishes (Asnol Petri Dish φ55 × 17 mm; AS ONE Corporation, Osaka, Japan) lined with two layers of filter paper (φ55 mm; Advantec Toyo Roshi Kaisha, Ltd., Tokyo, Japan), to which 2 mL of tap water containing 1 ppm iprodione (Rovral WP; Byer Crop Science K.K., Leverkusen, Germany) and a 2000-fold dilution of liquid fertilizer (Hyponex; Hyponex Japan, Ltd., Osaka, Japan) was added. For each cultivar, approximately 30 seeds were sown per Petri dish. The seeds were incubated for 10 days at 15 ℃ in a growth chamber under a 16:8-h L:D photoperiod. The 10-day-old seedlings were inoculated by spraying with a *B. lactucae* suspension ($1.0 \times 10^5$ conidia/mL). After inoculation, the Petri dishes were covered with their lids and incubated for an additional 10 days under the same conditions.

Disease reactions were assessed according to the criteria described by Nishiguchi and Futai (2010) and classified into four categories: sporulation, sporulation with necrosis, no sporulation with necrosis, and no sporulation.

## Evaluation of resistance and susceptibility in commercial lettuce

This experiment was conducted using the KKB001 isolate and was repeated twice for each variety. At that time, since we had not yet obtained the KKB002 isolate, we only evaluated the KKB001 isolate. Eighteen commercial lettuce varieties were sown in a commercial potting soil (Yosaku N-150) in 200-well seedling trays (1 seed/well× 10; each well: 25 mm diameter × 45 mm depth; Yanmar Holdings Co., Ltd., Osaka, Japan) and grown for 7 days in a growth chamber at 20°C under a 16:8-h L:D photoperiod. The seedlings were then sprayed with a *B. lactucae* suspension ($1.0 \times 10^5$ conidia/mL) until runoff and incubated overnight in a covered clear plastic box (Passetite TW-50, 250 mm × 356 mm × 100 mm; Gifu Plastic Industry Co., Ltd., Gifu, Japan) at 15 ℃ and 100% relative humidity in darkness for 8 h to facilitate infection.

After incubation, the lid was removed and the seedlings were maintained at 15 ℃ and 70% relative humidity under under a 12:12-h L:D photoperiod for an additional 6 days. After this period, the seedlings were sprayed with tap water and incubated overnight at 15 ℃ and 100% relative humidity to promote sporulation.

Disease incidence was assessed by evaluating each cotyledon or leaf for the presence of sporulation. Incidence was calculated as (number of sporulation plants / number of grown plants) × 100 (Fig. 1).

### Host range of B. lactucae in natural populations of Asteraceae plants

In total, three Asteraceae species representing six populations were tested for susceptibility to *B. lactucae*. After the above test, the KKB001 isolate was broken during the maintenance. Therefore, we only tested the KKB002 isolate. Seed samples of three *L. serriola* populations were collected in 2024 from Akita, Fukushima, and Hiroshima Prefectures. Seed samples from two populations of *Lactuca indica* and one population of *S. oleraceus* were collected in 2024 from Kagawa Prefecture.

The inoculation procedure followed the method used for the determination of *B. lactucae* races, as described above. Disease reactions were evaluated by assessing sporulation on cotyledons.

Additionally, 20 seeds of *L. serriola* collected from Hiroshima Prefecture were sown in commercial potting soil (Yosaku N-150) contained in 1/5000 Wagner pots and grown for 3 weeks in a greenhouse. The 3-week-old seedlings were then sprayed until runoff with a *B. lactucae* suspension ($1.0 \times 10^5$ conidia/mL). Inoculated seedlings were incubated overnight in a clear plastic box (Passetite TW-200, 250 mm × 356 mm × 325 mm) at 15 ℃ and 100% relative humidity in darkness for 8 h to facilitate infection. After incubation, the lid was removed, and the seedlings were maintained at 15 ℃ and 70% relative humidity, under a 12:12-h L:D photoperiod for 10 days. During this period, the seedlings were sprayed with tap water and incubated overnight at 15 ℃ and 100% relative humidity to promote sporulation. Conidia obtained from these inoculations were then used to infect new seedlings of *L. sativa* cv. Ciscoviva following the same method. Sporulation was evaluated on both cotyledons and true leaves.

## Results

# Morphology and taxonomy

Both isolates exhibited conidiophores that were straight with tree-like dichotomous branching, and candida that were hyaline and spherical (Table 1; Fig. 2). In isolate KKB002, conidiophores measured 207–476 × 9.8–13.8 µm, and conidia had a diameter of 16.2–17.8 µm. These morphological characteristics were consistent with previous descriptions (Kochman and Majewski 1970; Lee et al. 2021; Vieira and Barreto 2006).

For molecular characterization, nucleotide sequences of the LSU and cox2 fragments of both isolates were deposited in the DNA Data Bank of Japan (KKB001: LC778228 for LSU, LC778226 for cox2; KKB002: LC778229 for LSU, LC778227 for cox2). The LSU sequences of both isolates exhibited high homology with MF693907 (LSU of *B. lactucae*), and the cox2 sequences of both isolates were 100% identical to NC040179 (cox2 of *B. lactucae*) (data not shown).

### Determination of races and evaluation of resistance to commercial lettuce cultivars to B. lactucae

Twenty-five differential lettuce cultivars were examined for their susceptibility to the two *B. lactucae* isolates (Table 2). For isolate KKB001, 19 cultivars were susceptible, while six were not (R4T57D (*Dm4*), Nun Dm17 (*Dm17*), Argeles (*Dm38*), RYZ2164 (*Dm25*), Balesta, and Bellisimo). For isolate KKB002, only seven cultivars were susceptible (Green Towers (no R-gene), Valmaine (*Dm5/8*), LSE57/15 (*Dm7*), UC DM10 (*Dm10*), Capitan (*Dm11*), Hilde II (R12), UC DM14 (*Dm14*)). The resistance/susceptibility profiles of these two isolates differed markedly from those of races previously reported by the IBEB (2025) and Nishiguchi and Futai (2010) (Table 2), indicating that the present isolates represent new races of *B. lactucae*.

For KKB001, nine commercial cultivars exhibited sporulation on cotyledons or leaves. The remaining nine resistant cultivars showed little or no sporulation on cotyledons or leaves (Fig. 3).

### Host range of B. lactucae in Asteraceae plants in the wild

One cultivar of *L. sativa*, two *Lactuca* species, and one *Sonchus* species were inoculated with isolate KKB002. Sporulation was observed on the cotyledons of *L. sativa* cv. Ciscoviva and *L. serriola* collected from Hiroshima Prefecture. Sporulation on *L. serriola* seedlings was detected even in 1/5000 Wagner pots (Fig. 4a–c). Conidia obtained from these *L. serriola* seedlings were subsequently used to inoculate new seedlings of *L. sativa* cv. Ciscoviva, resulting in sporulation on cotyledons (Fig. 4d, e). This represents the first recorded instance of cross-infection of *B. lactucae* between *L. sativa* and *L. serriola* in Japan.

## Discussion

The genome of *L. sativa* has been sequenced and assembled, covering 2.4 Gb of the total 2.7 Gb, with these sequences organized into chromosomal pseudomolecules (Reyes-Chin-Wo et al. 2017). To date, 51 genes and 15 quantitative trait loci for resistance have been reported (Parra et al. 2016), and 27 *Dm* genes are located within major resistance clusters containing nucleotide-binding leucine-rich repeat genes (Christopoulou et al. 2015a, b). In addition, 11 *Dm* genes were reported more recently (Parra et al. 2021).

The gene-for-gene interaction between *L. sativa* and *B. lactucae* is now one of the best-characterized plant–pathogen systems (Hulbert and Michelmore 1985; Farrara et al. 1987; Ilott et al. 1987, 1989). Races of *B. lactucae* are defined based on their reactions on differential cultivars carrying specific

resistance genes. The newly identified races from Japan were distinct from those recorded by the IBEB and from races previously reported in Hyogo Prefecture. The IBEB primarily evaluates races collected from northwestern Europe and the western United States (IBEB 2025). The present findings indicate that *B. lactucae* races not considered by the IBEB are emerging in Japan.

One possible explanation is seed transmission. Although seed-borne *B. lactucae* has not been reported, infected seeds are known to serve as a major source of primary inoculum for other downy mildew pathogens, including *Peronospora effusa* (spinach) (Inaba et al. 1983), *Peronospora manshurica* (soybean) (Roongruangsree et al. 1988), *Peronospora halstedii* (sunflower) (Cohen and Sackston 1974), *Peronospora cristata* (opium poppy) (Montes-Borrego et al. 2009) and *Pseudoperonospora cubensis* (cucumber) (Cohen et al. 2014). Many commercial lettuce seeds supplied to Japan have been harvested from regions outside of the areas surveyed by the IBEB (Fig. 3), which could contribute to the emergence of novel races.

A second possible explanation is transmission via *L. serriola*. Wild populations of *B. lactucae* appear to play a major role in the epidemiology of downy mildew on cultivated lettuce, as *B. lactucae* isolates from *L. sativa* are thought to have emerged through several host shifts from *L. serriola* (Runge et al. 2021). Sexual reproduction is recognized as the primary mechanism generating new virulence phenotypes in *B. lactucae* (Michelmore and Ingram 1981); however, variation can also arise through asexual events such as recombination via anastomosis (Van Hese et al. 2016). These observations suggest that new races of *B. lactucae* are likely being produced on *L. serriola*.

The timing of the Invasion of *L. serriola*, the most dominant wild host, into Japan is unknown. Nonetheless, considerable variation in resistance to *B. lactucae* has been reported both among and within countries (Petrželová and Lebeda 2011). In the present study, sporulation was observed on the inoculated cotyledons of *L. sativa* cv. Ciscoviva and *L. serriola* (collected in Hiroshima Prefecture). Factors contributing to this variation include the ephemeral nature of this annual weed host, local ecological conditions, and agronomic management and weed control practices, which may influence the spatial structure of populations by affecting the survival of specific resistance-gene genotypes in the landscape (Petrželová and Lebeda 2011).

Thus, *L. serriola* may serve as the primary source of *B. lactucae* transmission in Japan. However, regular weeding is conducted throughout the country, including along field edges, suggesting that there are limited opportunities for encounters between *L. serriola* and cultivated *L. sativa*. Additionally, crop rotation, including with paddy rice, is common in lettuce fields in Kagawa Prefecture. Transmission by oospores is also possible, but oospore survival of *Peronospora destructor* is reduced when paddy rice is cultivated (Nishimura et al. 2022), implying that *B. lactucae* oospores may similarly be affected. Consequently, lettuce downy mildew may be less prevalent in Japan than in other countries. Nevertheless, continued evaluation of *B. lactucae* races in Japan remains important to anticipate the potential emergence of new races.

Finally, the resistant cultivars exhibited little or no sporulation on the cotyledons or leaves; thus, the use of resistant cultivars likely remains the most effective strategy for controlling *B. lactucae*. However, variety determining priorities are often driven by market demands for quality such as head form and cultivation suitability, as well as resistance to other major diseases, including Mirafiori lettuce big-vein ophiovirus and *Fusarium oxysporum* f. sp. *lactucae*.

## Declarations

The authors declare that they have no conflict of interest.

### Ethical approval

This article does not contain any studies performed with human participants or animals.

# Acknowledgments

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# Tables

Table 1 to 3 are available in the Supplementary Files section.

# Figures

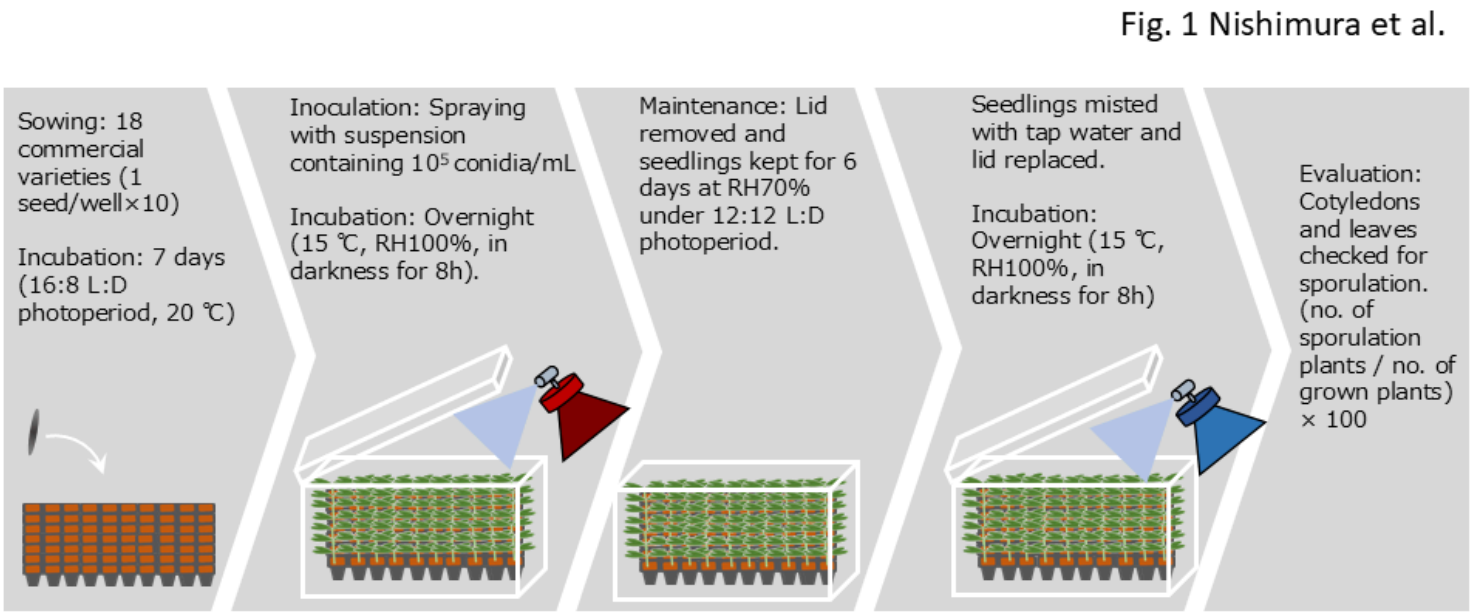
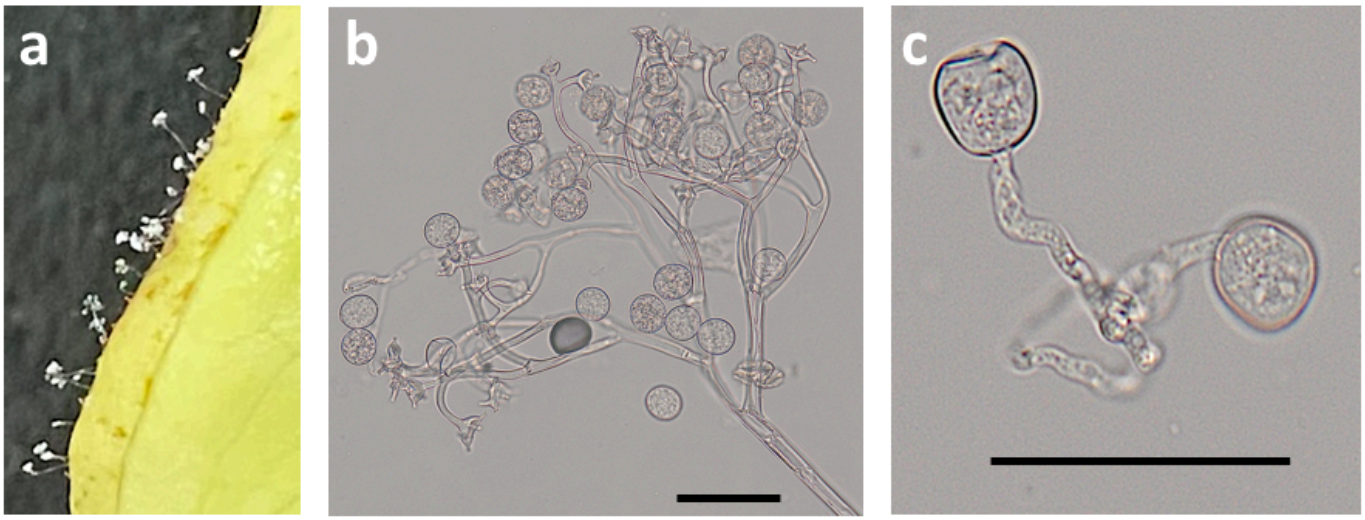



**Figure 1**

Methodology used to investigate the resistance and susceptibility of commercial varieties of *Lactuca sativa*to *Bremia lactucae*.

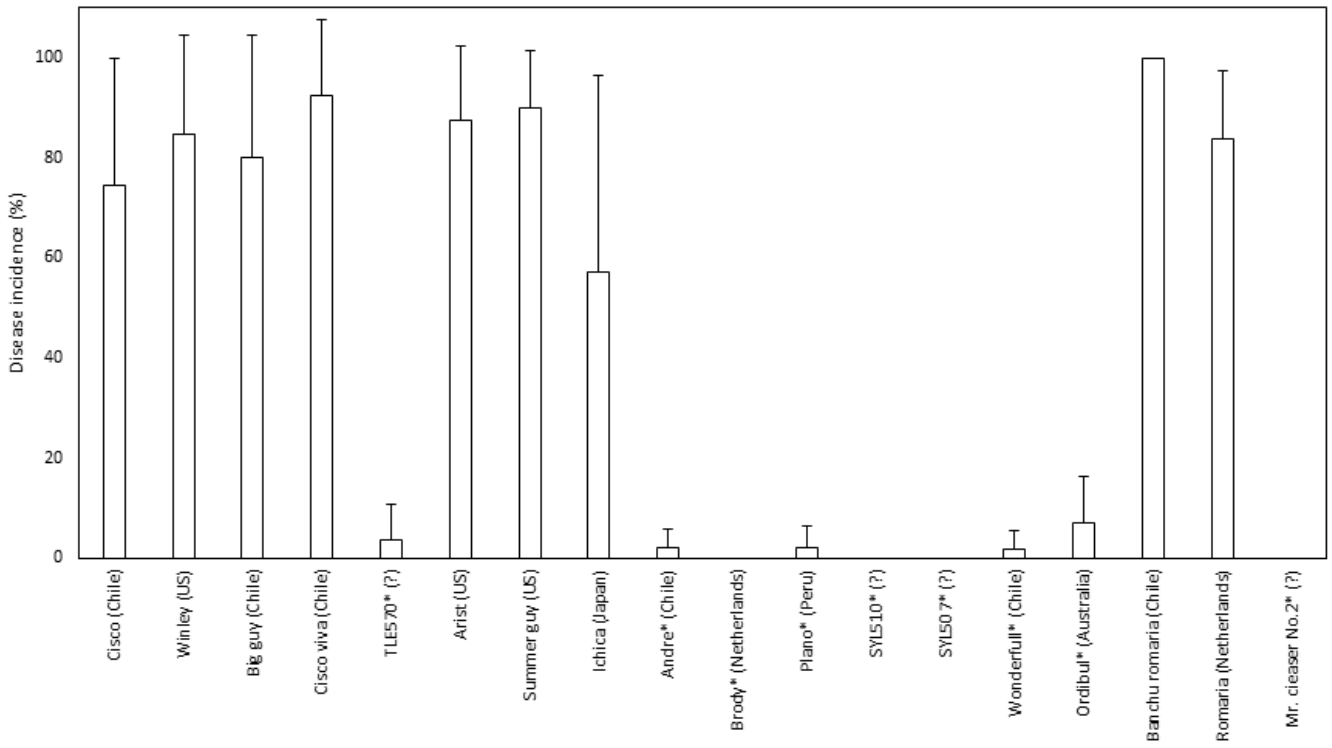
Fig. 2 Nishimura et al.




## Figure 2

Photomicrographs showing downy mildew caused by *Bremia lactucae* on *Lactuca sativa* collected from Kagawa Prefecture, Japan. (a) Sporulation on the leaf. (b) Conidiophore. (c) Germinating conidia with germ tube. Scale bars, 50 μm.

Fig. 3 Nishimura et al.




## Figure 3

Resistance of 18 commercial lettuce cultivars to a *Bremia lactucae* isolate collected from Kagawa Prefecture, Japan (KKB001). Parentheses indicate the country of origin of the seeds. Asterisks indicate cultivars that the seed company's website lists as resistant to downy mildew. Error bar indicates SD.

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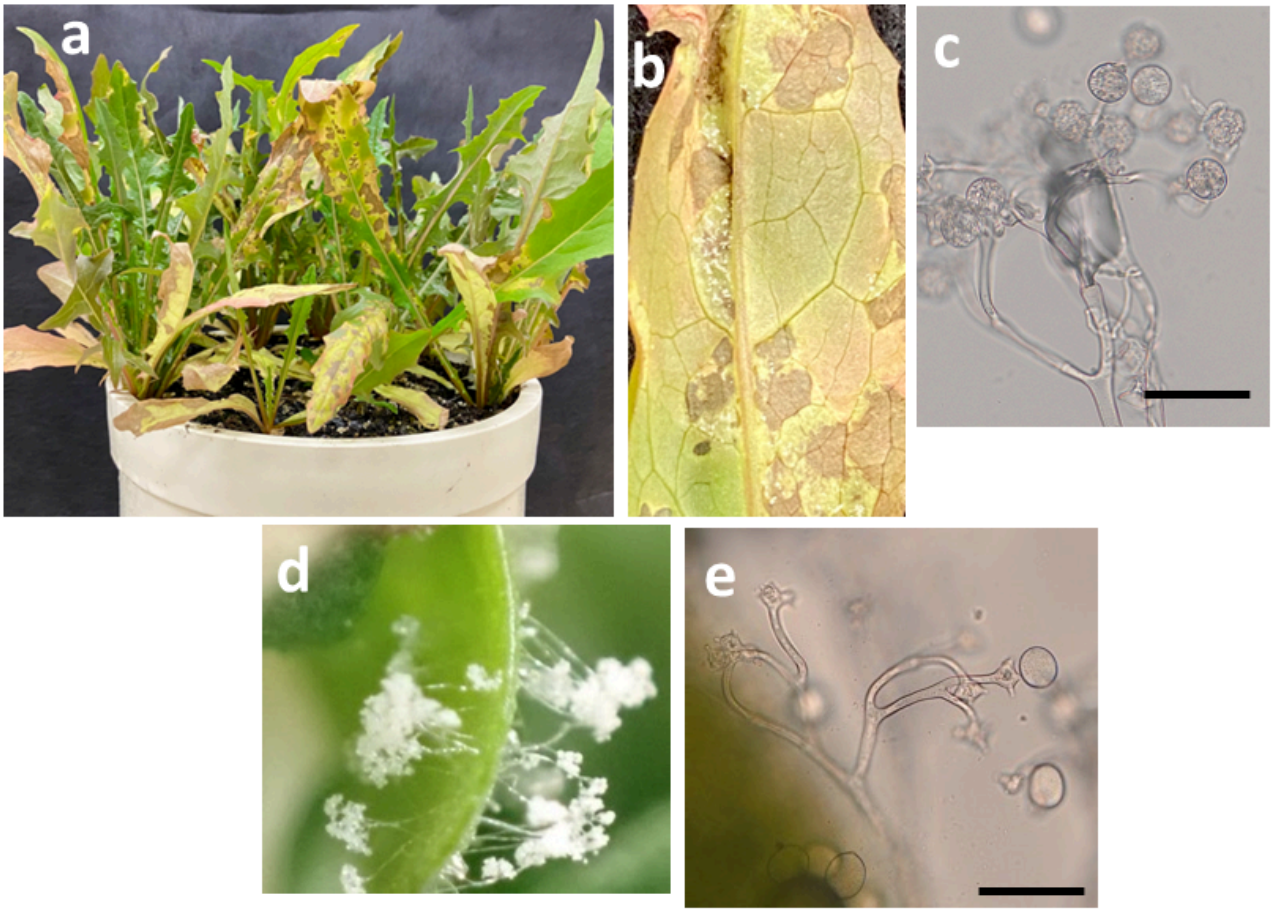


**Figure 4**

Symptoms and morphology of *Bremia lactucae*(KKB002) on *Lactuca serriola*, and cross-infection between *L. serriola*and *Lactuca sativa*. *Lactuca serriola* was collected from Hiroshima Prefecture, Japan. (a) Whole *L. serriola* plant. (b) Leaf (abaxial side) showing necrosis and sporulation. (c) Conidiophore of *B. lactucae* isolated from the *L. serriola* leaf shown in panel b. (d) Sporangia on the *L. sativa*cotyledon inoculated by panel b's sporangia. (e) Conidiophore of *B. lactucae*from *L. sativa* cotyledon. Scale bars, 50μm.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- 251002NishimuraTables.xlsx