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Perspective

Comprehensive Control System for Ginger Bacterial Wilt Disease Based on Anaerobic Soil Disinfestation

Mitsuo Horita [1,*], Yuso Kobara [1], Kazutaka Yano [2], Kazusa Hayashi [2], Yoshihide Nakamura [3], Kazuhiro Iiyama [4] and Tomoka Oki [2]

[1] Institute for Agro-Environmental Sciences, National Agriculture and Food Research Organization, Tsukuba 305-8604, Ibaraki, Japan
[2] Kochi Agriculture Research Center, Nankoku 783-0023, Kochi, Japan
[3] Nagasaki Agricultural and Forest Technical Development Center, Isahaya 854-0063, Nagasaki, Japan
[4] Faculty of Agriculture, Kyushu University, Fukuoka 819-0395, Fukuoka, Japan
* Correspondence: mhorita@naro.affrc.go.jp

Abstract: Bacterial wilt is a soil-borne disease that causes severe damage in ginger-growing regions of Japan (eight prefectures in the Shikoku, Kyushu, and Honshu regions). Because the pathogen *Ralstonia pseudosolanacearum* usually lives in deeper soil and infects host plants via the roots, it is not easy to eliminate even with chemical pesticides (such as soil fumigants). In our previous study, we found that anaerobic soil disinfestation with diluted ethanol (Et-ASD) effectively eliminated this pathogen. We conducted field experiments to confirm the effectiveness of Et-ASD in a ginger monoculture system. Eight trials were conducted in seven different ginger fields from spring to autumn. Excluding one trial in early spring, seven trials in summer successfully eliminated the pathogen from the field soil (below the detection limit by the developed sensitive bio-PCR method), and no disease recurrence was observed after ASD treatment. In addition, other useful methods for controlling the disease were explored, including proper field management after the disease outbreak and the disinfection of seed rhizomes. Based on these results, a comprehensive control system for bacterial wilt disease in ginger was developed.

Keywords: soil-borne disease; open field trial; disease suppression; proper field management; seed rhizome disinfection

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1. Introduction

Ginger (*Zingiber officinale* (Willd.) Rosc.) is a traditional and important crop for local farmers and industries in Japan. Outbreaks of bacterial wilt have been reported in the Kochi Prefecture, the largest ginger-producing region, and the affected area has expanded in recent years. The damage caused by this disease has also been reported in other areas, such as the Kyushu region, where ginger cultivation is thriving.

Few control tests for ginger bacterial wilt have been conducted, and no effective pesticides have been reported in Japan. At the production site, countermeasures have been taken, such as the early pull-out of diseased plants, and fallow crop rotation, such as non-host plants in diseased fields, but the fundamental problem (once the pathogen has invaded a field, it is difficult to eliminate completely) has not been solved.

In this review, first, we will describe the outbreak and damage of the disease in Japan, the characteristics of the disease, and the ecology of the pathogen. Then, we will explain our study on the assessment and development of (1) proper diagnosis and urgent treatment in diseased fields, (2) soil disinfection methods such as anaerobic soil disinfestation with low-concentration ethanol (Et-ASD), and (3) the seed rhizome disinfection method. Finally, we will discuss a comprehensive control system for ginger bacterial wilt that integrates the measures mentioned above. We will also discuss the potential drawbacks or risks of using Et-ASD or other methods for controlling bacterial wilt disease in ginger.

2. Bacterial Wilt Disease

Bacterial wilt disease caused by the phytopathogenic bacteria *Ralstonia* spp. occurs in crops such as tomatoes, eggplants, potatoes, ginger, and bananas and is a major problem in these growing regions worldwide [1]. This pathogen is divided into 5 races according to its host ranges [2], of which race 4 is specifically pathogenic to Zingiberaceae crops, such as ginger.

2.1. Outbreak of Bacterial Wilt Disease of Zingiberaceae Crops

In Japan, bacterial wilt disease occurs yearly in solanaceous crops, such as tomatoes and eggplants. Race 1 (infecting solanaceous crops, such as tomatoes, eggplants, tobacco, flowering plants, and various other plants) and race 3 (primarily affecting potatoes) strains have also been reported, but neither of these strains showed pathogenicity to Zingiberaceae crops [3]. Bacterial wilt disease caused by race 4 strains was confirmed in curcuma (*Curcuma alismatifolia*) cultivation fields in 1995, ginger fields in 1997, mioga (*Zingiber mioga*) fields in 1999, and turmeric (*Curcuma longa*, *C. aromatica*, and *C. zedoaria*) fields in 2014, and the pathogenicity and genetic characteristics of race 4 strains were different from those of the previously reported race 1 and 3 strains. Therefore, it has been suggested that race 4 strains may have been introduced from outside Japan with latently infected host plants (seed rhizomes) [4].

2.2. Damage Caused by Bacterial Wilt Disease Occurrences

Bacterial wilt in Zingiberaceae crops causes the entire plant to die and rot; thus, rhizomes of affected plants cannot be harvested and shipped, and the outbreak of this disease is directly linked to a decrease in shipments. In Kochi Prefecture, the largest ginger-producing region in Japan, bacterial wilt disease was first reported in 1997, and since then, the disease has occurred and expanded almost every year. The disease decreases crop yield by approximately 40%. The total decrease in the prefecture is estimated to be approximately 1400 t. The Kyushu region (Nagasaki, Kumamoto, Miyazaki, and Kagoshima) and Wakayama, Shimane, and Tochigi prefectures, where ginger is extensively cultivated, have also been damaged by bacterial wilt disease [4]. It is estimated that the total loss of income in Japan is approximately 1700 t, equivalent to USD 11 million.

In Okinawa Prefecture, the largest turmeric production area, bacterial wilt disease was first reported in 2014 [5]. It spreads to approximately 10% of the cultivated fields, and since the diseased fields have a yield decrease of approximately 80%, the total loss is estimated to be approximately 4.5 t. The damage is equivalent to approximately USD 0.2 million, a severe problem for turmeric production.

There have been few experimental studies on controlling bacterial wilt disease in Zingiberaceae crops, and no effective pesticides have been developed in Japan [6,7]. At the production site, countermeasures, such as early pull-out of the diseased plants and the surrounding plants and fallow or non-host plant (such as paddy rice and green manure) rotation in diseased fields, have been taken. Once a disease occurs in a field, the field is constantly threatened by the disease, and there are cases in which the farm is abandoned because no fundamental solution (elimination of the pathogen from the field soil) can be found.

2.3. Characteristics of Bacterial Wilt Disease

In plants infected with this pathogen, the lower 2–3 leaves first turn yellow and wilt. Then, the entire aboveground part turns yellow and wilts and finally lodges and dies (Figure 1A).

Subsequently, the disease spreads around the affected plants through water, such as rainfall and irrigation, and the plants develop and die one after another (Figure 1B), eventually spreading to the entire field and causing severe damage. This is a typical vascular disease; it usually spreads and moves to the ground after invading the host

roots. Subsequently, it occludes the vessel, reduces its ability to carry water, and causes withering symptoms.

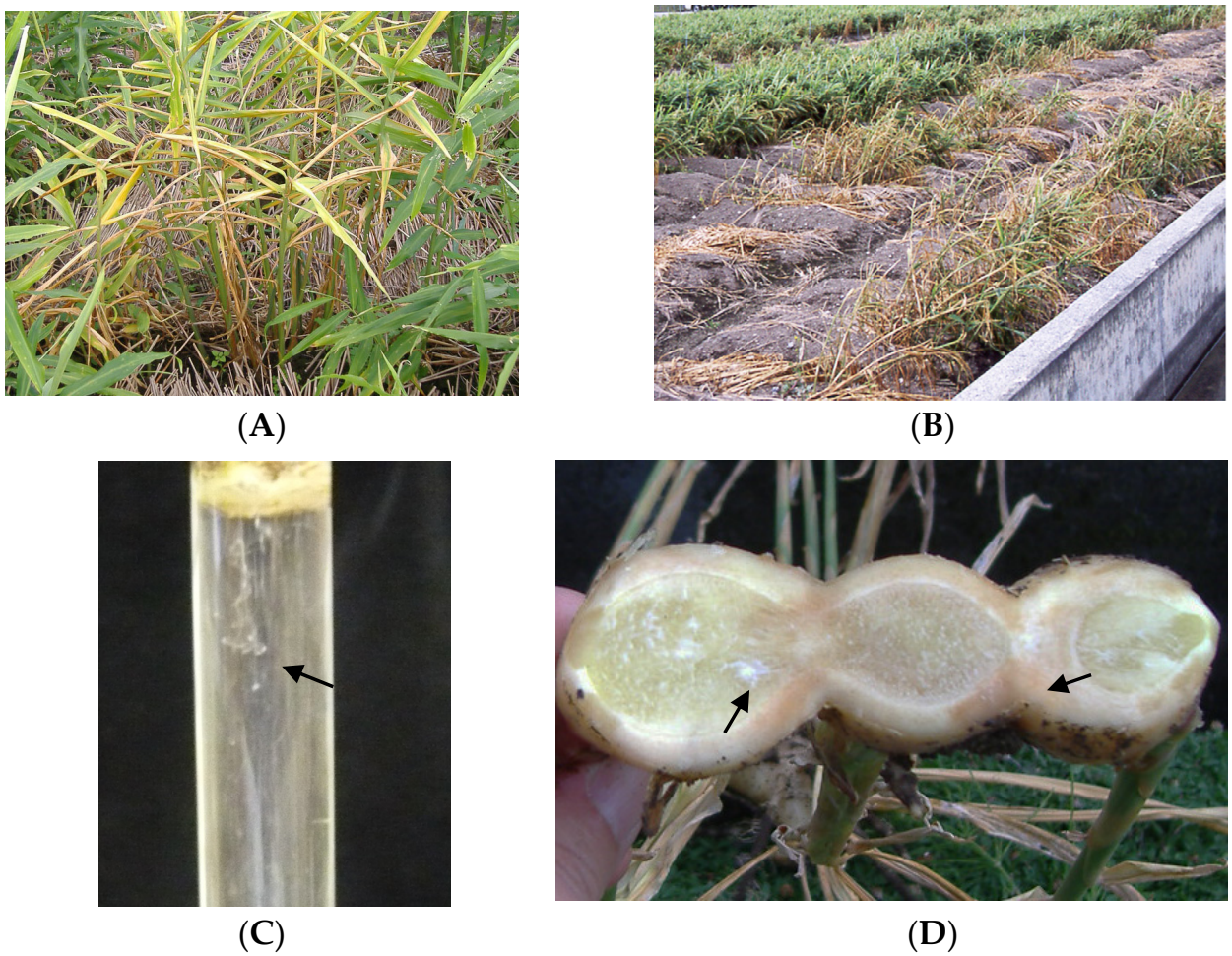

Figure 1. Disease symptoms and observable features of ginger bacterial wilt. The lower 2–3 leaves first turn yellow and wilt, and then the entire aboveground part turns yellow and wilts (**A**); the disease spreads around the affected plants, and the plants die one after another (**B**); when the cut surface of the diseased stem was immersed in a test tube containing water, bacterial ooze (arrow) overflowed from the inside and became creamy (**C**); and the rhizome of the diseased plant turned brown and rotted (arrow, right side), and a milky white bacterial mass (arrow, left side) was observed overflowing from the cut surface (**D**). These photos were taken at a diseased ginger (cultivar Tosa no. 1) field in Kochi Prefecture, Japan, in autumn.

As a characteristic of this disease, it can be seen from the cut surface of the diseased stem that the inside (vessel) turns brown. In addition, when the cut surface was immersed in a test tube containing water, bacterial ooze overflowed from the inside and became creamy (Figure 1C). The rhizome of the diseased plant turned brown and rotted, and a milky white bacterial mass was observed overflowing from the cut surface (Figure 1D).

Currently, phytopathogenic *Ralstonia* is divided into 3 species (*R. solanacearum*, *R. pseudosolanacearum,* and *R. syzygii*) [8], of which race 4 strains belong to *R. pseudosolanacearum*. The main pathogenicity determinant of this pathogen is the type III secretion system, which injects type III effector proteins into the plant cell for infection [9,10]. Based on whole-genome analyses of the race 4 strains [11–13], type III effector proteins (genes) that contribute to the specificity of Zingiberaceae crops were investigated [14].

2.4. Ecology of the Pathogen

Bacterial wilt is known as a soil-borne disease. This pathogen can survive in soil for a long time and infects roots as water moves through the soil and causes plants to wilt and die [15]. Then, the pathogen flows out with the surface soil during rainfall and irrigation treatments, thereby infecting neighboring plants at budding sites (that are susceptible to pathogen invasion as well as plant roots) and quickly spreading the disease (Figures 1B and 2) [16,17]. The wilted plants also release the pathogen from their roots and contaminate the soil. The disease caused by this pathogen is also known as a seed-borne disease. When seed rhizomes infected with this pathogen are planted, the rhizomes rot and do not germinate. Even if they germinate, they become diseased and rot later, causing the pathogen to flow out into the surroundings, causing soil contamination (Figure 2) [17].

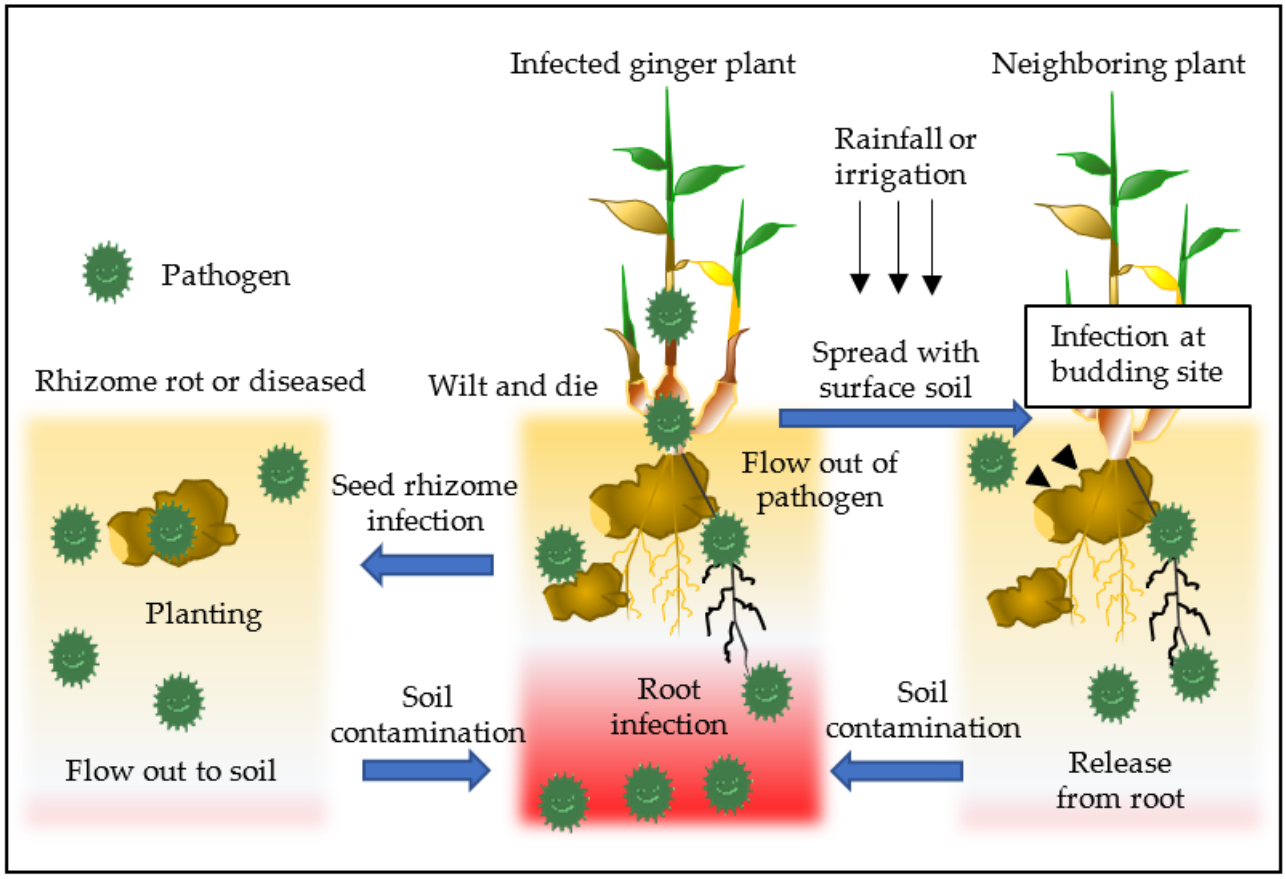

Figure 2. Infection cycle of the pathogen. This pathogen survives in the soil, infects the plant roots, and causes wilting and death (see (**center**) side). Then, the pathogen flows out with the surface soil during rainfall and irrigation (arrow), thereby infecting neighboring plants at budding sites (arrowhead) and causing wilting. The wilted plants also release the pathogen from their roots and contaminate the soil (see (**right**) side). When infected seed rhizomes are planted, the rhizomes become rotten or diseased, and the pathogen flows out into the soil (see (**left**) side).

3. Diagnosis/Control System

Regarding the diagnosis of and control measures against this disease, the authors proposed to divide them into three main situations: "disease occurrence," "soil disinfestation measure," and "seed rhizome measure" (Figure 3), and the details of each are explained below.

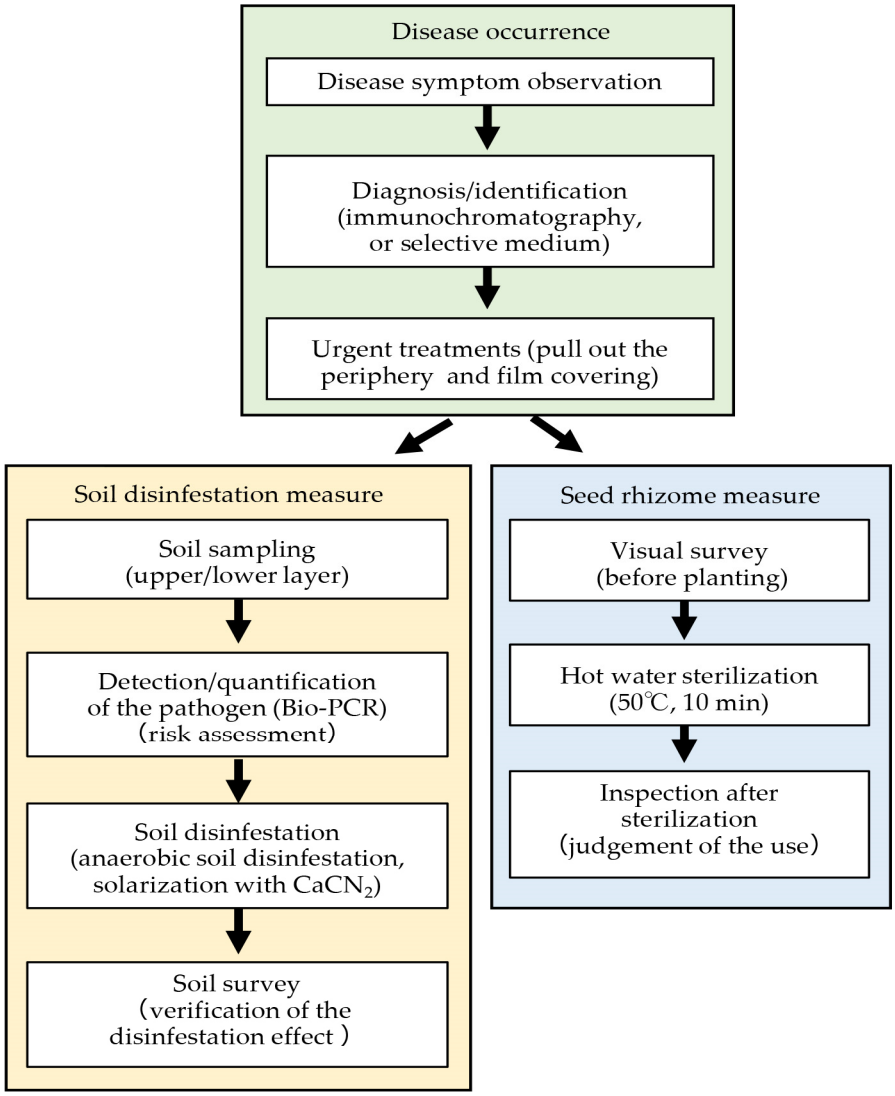

Figure 3. Control system for ginger bacterial wilt disease. This system is divided into three main situations: disease occurrence (diagnosis, identification of the pathogen, and urgent treatment); soil disinfestation measure (soil survey using bio-PCR and soil disinfestation treatment such as anaerobic soil disinfestation); and seed rhizome measure (visual survey and hot-water sterilization). The details of each are described below.

3.1. Disease Occurrence

If potentially diseased ginger plants showing any bacterial wilt-like symptoms (leaves turn yellow and wilt, inside of stem turns brown, inside of rhizomes turn brown and rot, or overflow of bacterial ooze from the cut surface of the stem or rhizome) (Figure 1A–D) are found in the field, diagnosis of the plant using immunochromatography (Agdia ImmunoStrip for Ralstonia solanacearum, Agdia Inc., Elkhart, IN, USA) or selective isolation of the pathogen using a semi-selective agar medium (modified SMSA medium) [18] from the grounded plant or the effluent is needed. If this symptom is diagnosed as bacterial wilt disease, all diseased plants and 2 m of the surroundings should be pulled out and the soil surface covered with a plastic film until harvesting so that it is not directly exposed to rainfall and irrigation immediately (Figure 4). If countermeasures are delayed, there is a high risk that the pathogen will flow out together with the soil during rainfall and irrigation treatments, thereby infecting neighboring plants at budding sites (that are susceptible to pathogen invasion as well as plant roots) and quickly spreading the disease (Figure 2) [16].

Figure 4. Urgent treatment in a diseased field. The diseased plants and the surrounding plants were pulled out and the soil was covered with a film immediately, because otherwise the pathogen will flow out together with the surface soil during rainfall and irrigation from the diseased plants, thereby infecting neighboring plants and quickly spreading the disease (Figures 1B and 2).

3.2. Soil Disinfestation Measure

In fields where bacterial wilt occurred, the distribution and density of pathogenic bacteria in the soil were determined. Soil should be collected from multiple sites where the disease occurred recently and in the past, along with the surroundings (Table 1), since this bacteria can survive in the soil for a long time. In addition, because the bacterial wilt disease pathogen often invades and survives deep in the soil, not only the upper soil layer (0–30 cm) but also the lower layer (30–60 cm) should be collected.

Table 1. Bacterial wilt pathogen concentration in a diseased ginger field (example).

Sampling Site		Pathogen (cfu/g Soil)	Disease Occurrence
1	0–30 cm	3.9×10^4	Occurred
	30–60 cm	4.3×10^4	
2	0–30 cm	1.0×10^2	Occurred in the past
	30–60 cm	ND	
3	0–30 cm	ND	Not occurred
	30–60 cm	ND	
4	0–30 cm	ND	Not occurred
	30–60 cm	ND	

ND: below detection limit (<1 cfu/g soil). Bacterial wilt pathogen (*Ralstonia pseudo-solanacearum*) concentration in a diseased ginger field (Tosa, Kochi Prefecture, Japan) after cultivation (November 2016) was assessed by bio-PCR method. Each sampling site was at least 10 m apart. The pathogen was detected on diseased sites in the past (no. 2) as well as the recently diseased site (no. 1).

To detect pathogens in the field soil, we designed race-4-specific nested PCR primer sets based on whole-genome analyses of race 4 strains [11] and developed a highly sensitive bio-PCR method (>1 colony-forming unit (cfu)/g soil) [19]. By combining this with a statistical method (most probable number method), the number of pathogens in the soil can be quantified at the same time [20,21]. The bio-PCR method has relatively higher detection sensitivity compared with the method using a selective medium which was commonly used for the isolation of the pathogen.

When the pathogen is detected in the soil, soil disinfestation measures should be performed, such as (1) Et-ASD (recommended for flat land) and (2) solar soil disinfestation using calcium cyanamide (suitable for sloping land or a field that cannot supply a steady amount of irrigation water) (Figure 3). When soil disinfestation is performed, it is necessary to abandon ginger cultivation because the proper disinfection period (summer) overlaps with the cultivation period.

3.2.1. Experiences with Et-ASD in Managing Bacterial Wilt in Ginger

In our experiences with Et-ASD, ethanol was diluted to a concentration of approximately 0.6–1% (*v/v*) using a liquid fertilizer mixer or power sprayer, and the soil was irrigated (approximately 60–100 L/m^2; the amount of water required varies depending on the type of soil) using irrigation tubes. After irrigation, the soil surface was covered with a plastic film (for over a month) to prevent air from entering. Based on previous research, soil microorganisms consume oxygen and become anaerobic. Thereafter, volatile organic acids, such as acetic acid, and metal ions, such as Fe^{2+}, accumulate and kill plant pathogens (bacteria, fungi, nematodes, and weeds) in the soil [22–24]. Ethanol, together with water, permeates deep layers (approximately 1 m) and creates an anaerobic environment; therefore, it has a disinfestation effect against plant pathogens that live in the lower layers. This method is the most effective in the summer when the soil temperature is consistently above 25 °C. In addition, because a large amount of irrigation water is required, the field may be difficult to treat depending on its conditions (soil water retention capacity, irrigation equipment, and slope).

Our previous studies are shown in Table 2. We have conducted Et-ASD experiments (8 trials) at 7 different open ginger fields (400–1700 m^2 wide), which were located in the major ginger production area in the midwestern part of Kochi Prefecture (Figure 5), from spring to autumn in 2016–2019. The irrigation water volume was determined based on a three-phase (liquid, air, and solid) analysis of the soil and its type. As a result, 60–96 L/m^2 of water was irrigated, and the ethanol concentration ranged from 0.5 to 1.0% (*v/v*). The earliest removal of the plastic covering occurred after 54 days.

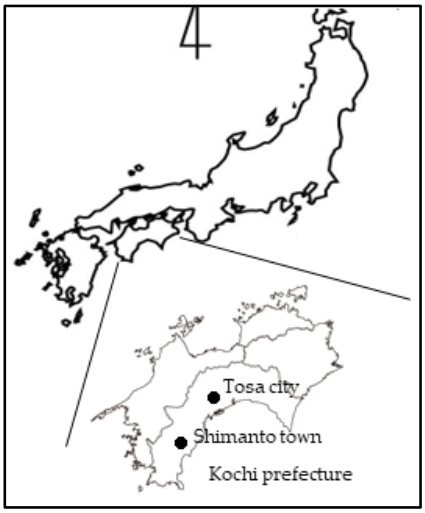

Figure 5. Location of anaerobic soil disinfestation experiment field (Tosa city and Shimanto town in Kochi Prefecture, Japan). Details of each experiment are shown in Table 2.

As an example of the Et-ASD test, the results of trial 6 (Table 2) at Tosa City (from July 25th to December 11th in 2018, 0.69% (*v/v*) ethanol dilution, and 93.6 L/m^2 irrigation) are shown in Table 3 and Figure 6A,B. Soil temperatures at 20 and 50 cm soil depths were kept over 30 °C for 6 weeks (Figure 6A). The redox potential (Eh) and soil temperature were measured using Eh meters at 20 cm and 50 cm soil depths. The anaerobic conditions (<200 mV) were enhanced and maintained for longer than 2 weeks (Figure 6B). Populations of *R. pseudosolanacearum* strains in the upper (0–30 cm) and lower (30–60 cm) soil depths before and after Et-ASD were assessed. Before treatment, this pathogen resided in the lower soil layers. After Et-ASD treatment, pathogen densities at all sampling sites decreased to below the detection limit (<1 cfu/g soil) (Table 3). After the Et-ASD treatments, ginger cultivation was resumed in this field in the following March. The disease occurrence (number and rate of diseased plants) during cultivation was observed monthly from the

corner of the field using a telescope. If suspicious plants appeared, we entered the field and checked whether this was bacterial wilt or not. As a result, no bacterial wilt disease occurred during the cultivation period (until late November in 2019). Additionally, ginger has been cultivated since 2020, and disease outbreaks have not yet been reported (Table 2).

Table 2. Et-ASD trials at open fields in Kochi Prefecture, Japan.

No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Location (soil type)	Tosa (red-yellow soil)	Tosa (red-yellow soil)	Tosa (gray lowland soil)	Tosa (gray lowland soil)	Tosa (red-yellow soil)	Tosa (gray lowland soil)	Shimanto (brown lowland soil)	Tosa (gray lowland soil)
Test area (m^2)	1000	400	1000	1700	600	800	1000	1400
Start date	27 July 2016	9 September 2016	10 March 2017	23 August 2017	17 July 2018	25 July 2018	8 August 2018	9 August 2019
End date	21 December 2016	21 December 2016	2 May 2017	19 October 2017	5 October 2018	11 December 2018	3 October 2018	16 October 2019
Irrigation tube spacing (m)	1.7	0.7	0.9	0.9	2.5	1.3–1.7	0.9	1.2
Ethanol supplied (L)	390	182	390	650	312	520	520	728
Pre-irrigation (L)	18,400	Un known	24,000	40,000	1500	48,500	19,723	49,000
Ethanol diluent irrigation (L)	44,000	24,000	53,000	90,000	41,715	26,380	60,171	33,000
Total amount of irrigation (L)	62,400	24,000	77,000	130,000	43,215	74,880	79,894	82,000
Total amount of irrigation (L m^{-2})	62.4	60	77	77	72	93.6	79.9	58.6
Ethanol conc. (*v*/*v*, %)	0.63	0.76	0.51	0.50	0.72	0.69	0.65	0.89
Time required (h)	5	Unknown	3.5	6	6	8.5	6	10.5
Covering film (thickness)	PVC (0.075 mm)	PE + PVC	PVC (0.05 mm)	PE + PVC (0.05 mm)	PVC	PE + PVC	PE + PVAC	PE + PVAC
Pathogen in soil (after ASD)	Not detected	Not detected	Survived	Not detected	Not detected	Not detected	Not detected	Not detected
Remarks (field condition after ASD)	No disease occurrence after 4 works (2017–2020)	No disease occurrence (2017)	Re-try ASD in next summer season (see no. 4)	Disease occurred (single plant in 2018). No disease occurrence thereafter (2019–2020)	No disease occurrence (2019–2020)	No disease occurrence (2019–2020)	No disease occurrence (2019)	No disease occurrence (2020)

PVC: polyvinyl chloride, PE: polyethylene, PVAC: polyvinyl acetate. Anaerobic soil disinfestation with low-concentration-ethanol (Et-ASD) experiments (8 trials) at 7 different open ginger fields in Kochi Prefecture (Figure 5) were conducted from spring to autumn in 2016–2019. Each experiment condition is indicated. After Et-ASD experiments (7 trials) in the summer, pathogen densities at all sampling sites decreased below the detection limit (>1 cfu/g soil). In the case of Et-ASD in early spring (trial 3), the pathogen survived. Ginger cultivation after ASD in the test fields was resumed, and the disease recurrence was suppressed.

Similarly, in other Et-ASD treatments in the summer season (6 trials), the development of anaerobic conditions and the maintenance of high soil temperatures (>25 °C) during the

treatments were confirmed (data not shown). After the Et-ASD treatment, the pathogen densities at all sampling sites decreased below the detection limit (Table 2).

Table 3. Bacterial wilt pathogen concentration before and after Et-ASD in field soil.

Sampling Site		Pathogen (cfu/g Soil)	
		Before ASD	After ASD
1	0–30 cm	3.3×10^2	ND
	30–60 cm	1.0×10^2	ND
2	0–30 cm	<33	ND
	30–60 cm	ND	ND
3	0–30 cm	1.3×10^3	ND
	30–60 cm	33	ND
4	0–30 cm	2.7×10^2	ND
	30–60 cm	1.1×10^3	ND

ND: below detection limit (<1 cfu/g soil). Bacterial wilt pathogen (*Ralstonia pseudosolanacearum*) concentration before and after anaerobic soil disinfestation with low-concentration-ethanol (Et-ASD) treatment in field soil (trial 6 in Table 2) was assessed by Bio-PCR method. Before treatment, this pathogen resided both in the lower and upper soil layers. After Et-ASD treatment, pathogen densities at all sampling sites decreased to below the detection limit.

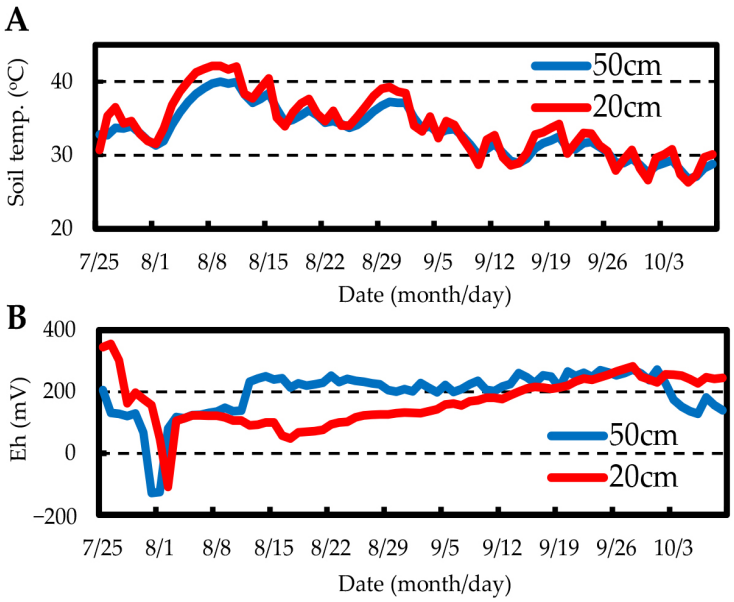

Figure 6. Soil temperature (**A**) and redox potential (Eh) (**B**) during anaerobic soil disinfestation treatment (trial 6 (Table 2) at Tosa city from July 25th to December 11th in 2018). Soil temperatures at 20 and 50 cm soil depths were kept over 30 °C for 6 weeks (**A**). The anaerobic conditions (Eh was below 200 mV) at 20 and 50 cm soil depths were maintained for longer than 2 weeks (**B**).

Conversely, the soil temperature of the early-spring Et-ASD treatment (from 10 March to 2 May) remained under 20 °C at all times during the treatment, and the pathogen at some sampling sites survived (trial 3 in Table 2). Therefore, the Et-ASD treatment was repeated the following summer, and the pathogen at all sampling sites decreased to below the detection limit (trial 4 in Table 2).

After that, ginger cultivation in the test fields was resumed. No disease recurrence has been reported (except in trial 4). These results indicate that Et-ASD treatment during the summer might be a promising disinfestation practice to suppress ginger bacterial wilt pathogens in the lower soil layer.

3.2.2. Solarization Using Calcium Cyanamide

We recommend solarization using calcium cyanamide ($CaCN_2$) when the amount of irrigation water (60 L/m^2 or more) required for the ASD cannot be secured or when irrigation treatment cannot be performed on slopes (slopes of 6–7% or more) [17]. Based on our experiences, in hot seasons (minimum soil temperature is 25 °C or higher), calcium cyanamide (200 kg/1000 m^2) is mixed with green manure (such as cut-down sorghum plants) and plowed into the soil to promote the decomposition of organic matter. After irrigation (less than 40 L/m^2), it is covered with a plastic film for 2 months or more for solar disinfection with high humidity. Soil microorganisms utilize decomposed organic matter and consume oxygen. As a result, an anaerobic disinfestation effect was expected. Our test in some ginger fields confirmed its disinfestation effect against the bacterial wilt pathogen living in the deep layer of the soil [25].

Chen et al. [26] reported that fumigation with calcium cyanamide efficiently suppressed soil-borne disease and increased tomato growth and yield since calcium cyanamide is a combination of fumigant and fertilizer (subsequently converted to ammonium).

3.2.3. Economic Analysis

We conducted an economic analysis for our field trials (Table 4). In open fields where bacterial wilt has occurred, if the yield decreases by 30% or more, the balance will become red, and it is possible that the pathogen will spread through the soil and the damage will continue and expand. If the outbreak of bacterial wilt disease is suppressed by taking "soil disinfestation measures," it is estimated that the income rate can be recovered even if the cost of these measures is added to the operating expenses [17].

Table 4. Economic analysis of soil disinfestation measures (per 1000 m^2).

	No Countermeasures	**Et-ASD (No Recurrence) ***		**Solarization with $CaCN_2$ (No Recurrence)**
	(30% Loss)	**(1st Work)**	**(2nd Work)**	**(1st Work)**
Average yield (kg)	3206	4580	4580	4580
Gross revenue (USD)	8010	11,450	11,450	11,450
Operating expenses (USD)	8020	9890	7620	8920
(Material for soil disinfestation)	400	1284	0	314
(Related materials for soil disinfestation)	0	986	0	986
(Others)	7620	7620	7620	7620
Income (USD)	−10	1560	3830	2530
Income rate (%)	0	14	33	22

* Disease suppression effect was confirmed up to the 2nd work after soil disinfection. Gross revenue was calculated as 2.5 USD/kg of ginger rhizomes. Material expense for Et-ASD was calculated as 70 t of 0.75% (*v*/*v*) ethanol dilution (2.44 USD/L 99.5% ethanol) irrigation. Material expense for solarization with $CaCN_2$ was calculated as 200 kg of $CaCN_2$ treatment (1.57 USD/kg). Expenses such as the covering film, irrigation tube, etc., were accounted as related materials for soil disinfestation. In the case of no countermeasures, the expense for the chemical (dazomet, 40 kg) to control soil pests other than bacterial wilt pathogen was included.

3.3. Seed Rhizome Measure

In the case of ginger, the seed rhizomes were usually preserved in plastic containers with field soil on them and kept at a low temperature (around 15 °C) and high humidity (90% or more). If there was a possibility of seed rhizome contamination, such as bacterial wilt disease in the vicinity of the field where the seed rhizomes were collected, or if the origin of the seed rhizomes distributed in lots was unknown, a visual survey was conducted before planting. Seed rhizomes with abnormalities (scratches, rot, discoloration, mold growth, etc.) were removed, and only rhizomes that looked healthy were sterilized with

hot water (50 °C, 10 min treatment) [27,28]. This seed rhizome disinfection method was effective against bacterial wilt pathogens living around the rhizomes as well as the ginger root rot disease pathogen *Pythium myriotylum* [29]. If the temperature was too high, the germination rate decreased, whereas if the temperature was too low, a sufficient sterilization effect was not obtained. After disinfection, the presence or absence of pathogenic bacteria was investigated to determine whether the seed rhizomes should be used (Figure 3).

4. Limitations and Challenges of the Study for the Proposed Control System

Chemical pesticides (fumigants) and resistant varieties (grafting) are common measures against bacterial wilt [6,7]. However, fumigants are effective up to the upper soil layer but not in the lower layer. Except for a few wild Zingiberaceae plants, resistant cultivars have not yet been reported [30,31]. For that reason, disease-free seed rhizome preparation and cultivation in isolated areas in Australia [32] and Hawaii [33] and bio-fumigation with the plants possessing bactericidal compounds [31,33] have been considered and carried out. We think a limitation of these measures is that no fundamental solution (elimination of the pathogen from the infested field) can be found. In recent years, the effectiveness of ASD technology has been evaluated, and it has been used as a countermeasure against bacterial wilt disease [34–41]. Our ASD test validated that this is an effective countermeasure against ginger bacterial wilt disease (particularly in open fields), and this technology is expected to expand in the future.

In this review, we introduced our previous ASD tests using low-concentration ethanol. ASD treatments using other carbon sources such as rice bran (solid material) and molasses (liquid) against bacterial wilt pathogens have been previously reported in Japan [34,40,41]. When rice bran is used, pre-mixing it into deeper soil (>40 cm) with deep tillage rotary is needed [40]. In the case of molasses, which is highly viscous and not easy to handle, the ASD effect using this material is sometimes unstable even if the soil permeability is uneven [34,41]. Conversely, ethanol has low viscosity and possesses relatively higher soil permeability, and ASD using ethanol is relatively unaffected by soil conditions compared with other materials. However, ASD's effect at low temperatures is unstable against bacterial wilt pathogens living in the deep layer of the soil. Therefore, we recommend ASD treatment to Japanese farmers during hot seasons. It is necessary to improve this method so that the effect is stable even at low temperatures.

To improve ASD treatment, the elucidation of its disinfestation mechanism is needed. It is speculated that many factors (soil microbial community, additive organic matter, temperature, and the physical and chemical properties of the soil, among others) affect the success of ASD [42–50]. During ASD treatment, specific soil microorganism groups which belong to *Clostridium* spp. in the phylum Firmicutes have been reported when any carbon sources were applied [42–47]. Some species and strains belonging to this genus are known as volatile organic acid (such as acetic acid, butyric acid, and caproic acid) producers. These products are effective at disinfesting soil-borne pathogens including the phytopathogenic *Ralstonia* spp. [24,36,42,48]. Hewavitharana et al. [49] reported that these kinds of materials were produced at comparatively high temperatures and suggested that this has a correlation with an increase in populations of *Clostridium* spp. It is also known that the degree of disinfestation differs among soil types [50], even though the same carbon sources were applied for ASD, and the correlation of soil type with the multiplication of *Clostridium* spp. should be analyzed.

When considering a comprehensive control system for bacterial wilt disease in ginger, combining many techniques and options is considered effective instead of relying on ASD techniques alone. In the present study, it was considered effective to take emergency measures in the event of disease outbreaks and prevent the introduction of contaminated ginger rhizomes. Combining these measures with more effective measures is expected to prevent the spread of the disease.

Seed rhizome disinfection with hot water is available for rhizome surface sterilization but not inside [27,29]. Therefore, we recommend this method for seed rhizomes considered

to be less infected. It is necessary to improve this method or search for other useful chemicals. Pulling out diseased plants and their surroundings and then using film coverings is available for urgent treatment in diseased fields. If the discovery of diseased plants is delayed and too many plants are diseased, the range of plants to be removed becomes enormous. To solve this problem, we are considering the utilization of drone aerial photography that can identify a wide range of diseased plants in a short time.

This can be used not only for bacterial wilt disease of ginger crops but also as a countermeasure against the disease of solanaceous crops and other soil-borne pathogens.

5. Conclusions

A comprehensive control system for ginger bacterial wilt disease based on soil disinfection measures such as ASD in Japan was reviewed. Et-ASD treatment in the summer effectively eliminated the pathogen from field soil and suppressed the occurrence of the disease. It was considered effective to take emergency measures in the event of disease outbreaks and prevent the introduction of contaminated ginger rhizomes. Combining these measures is expected to prevent the spread of the disease.

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