2018 International CLUBROOT Workshop

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CANADA

Resting spores of clubroot detection service by LAMP method in Japan

vegetalia, inc.

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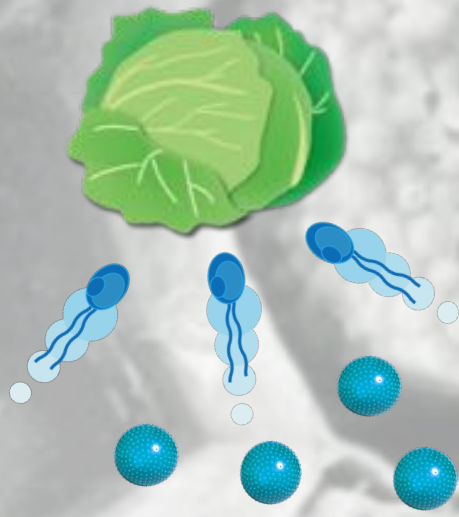
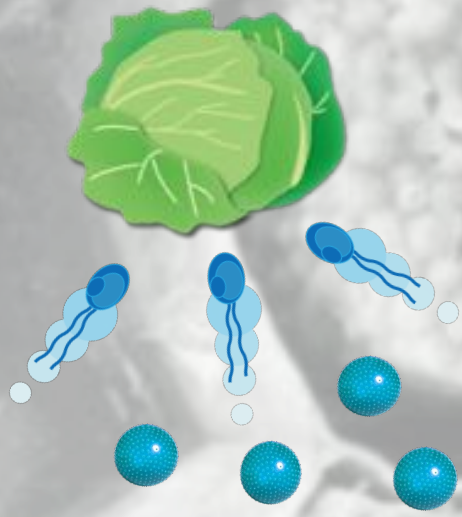
Outline



1. Introduction
2. Loop-mediated Isothermal Amplification (LAMP)
3. Club root Density Measurement Service in Japan
4. Conclusion

Outline



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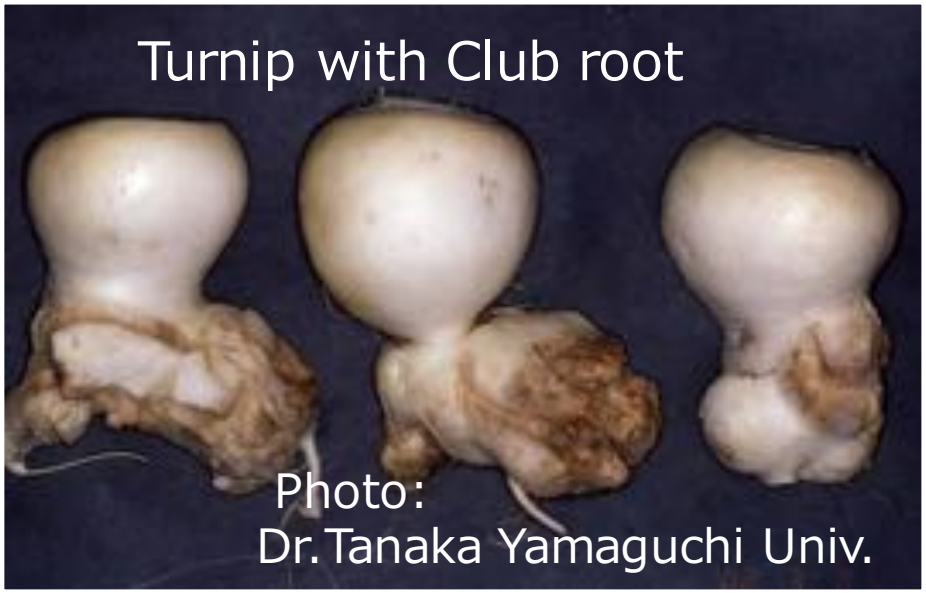
Clubroot disease in the Brassica family

Plasmodiophora brassicae

Chinese cabbage field that withered or dead with Club root

Chinese cabbage with Clubroot

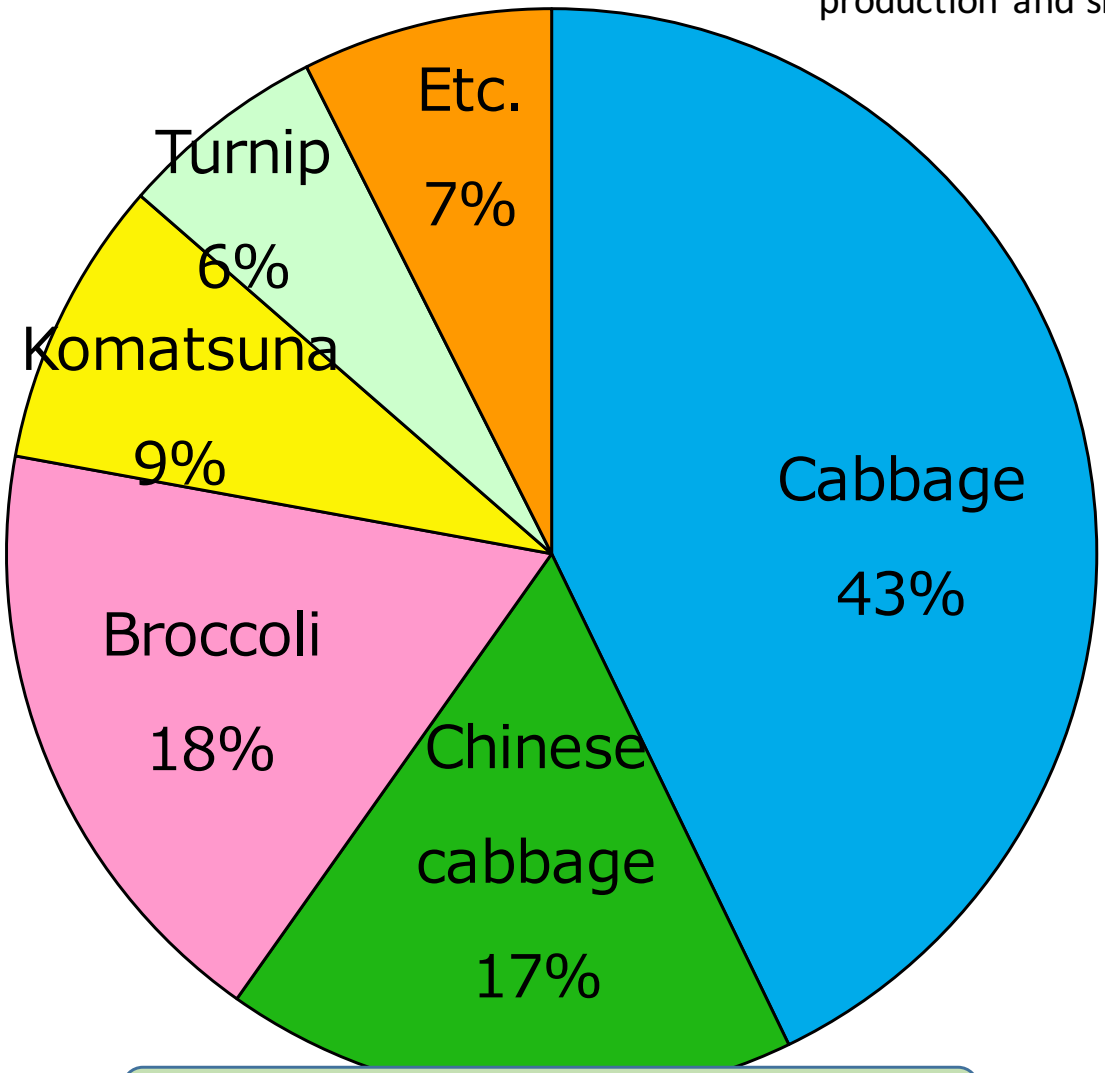


Turnip with Club root

Photo:
Dr. Tanaka Yamaguchi Univ.

Risk of Clubroot disease Cultivation area

Ministry of Agriculture, Forestry and Fisheries: From the statistics on production and shipment of vegetables produced in 2016 (vegetables)



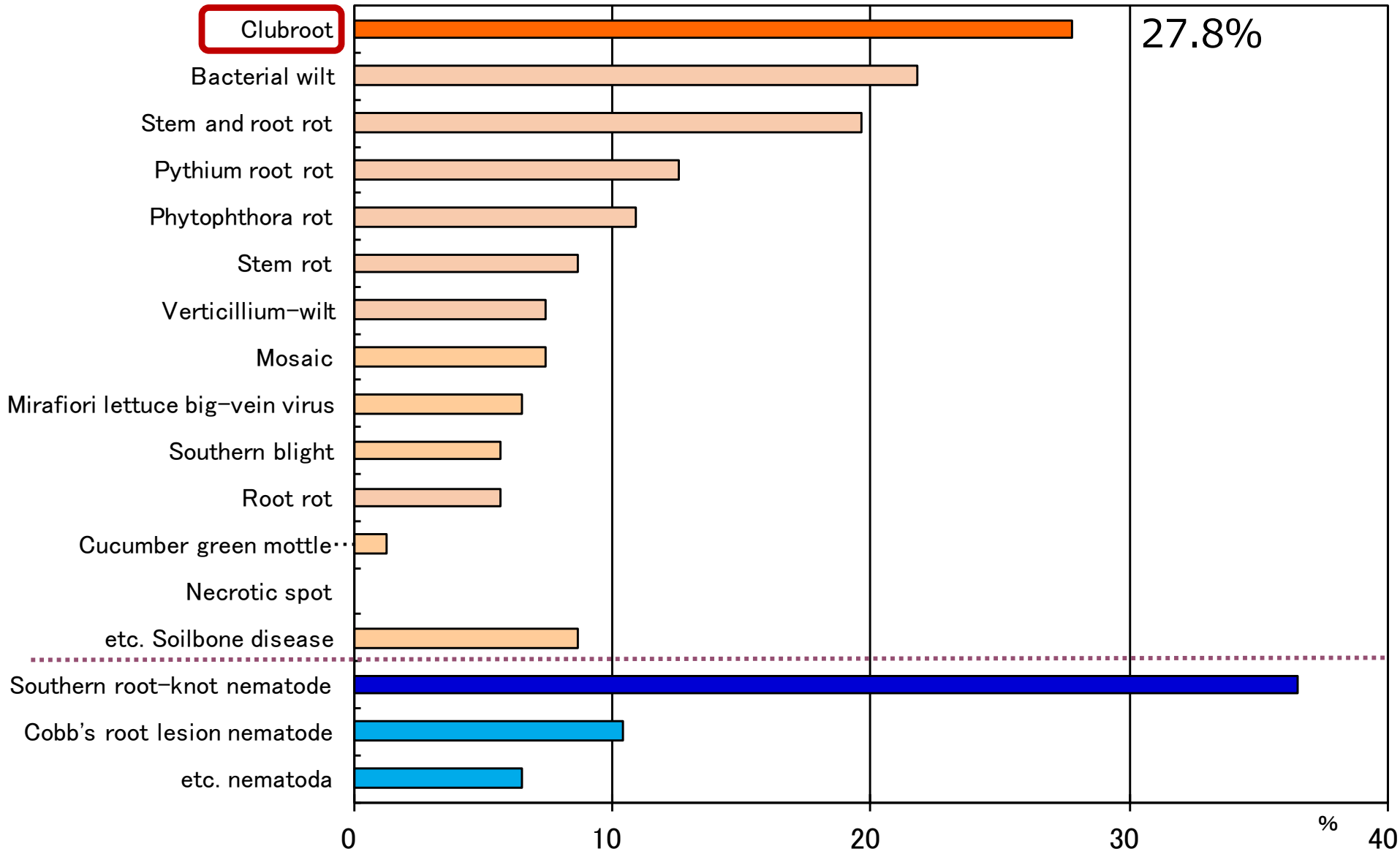
Total cultivation area : 80,772ha

Brassica family vegetable are important in Japan

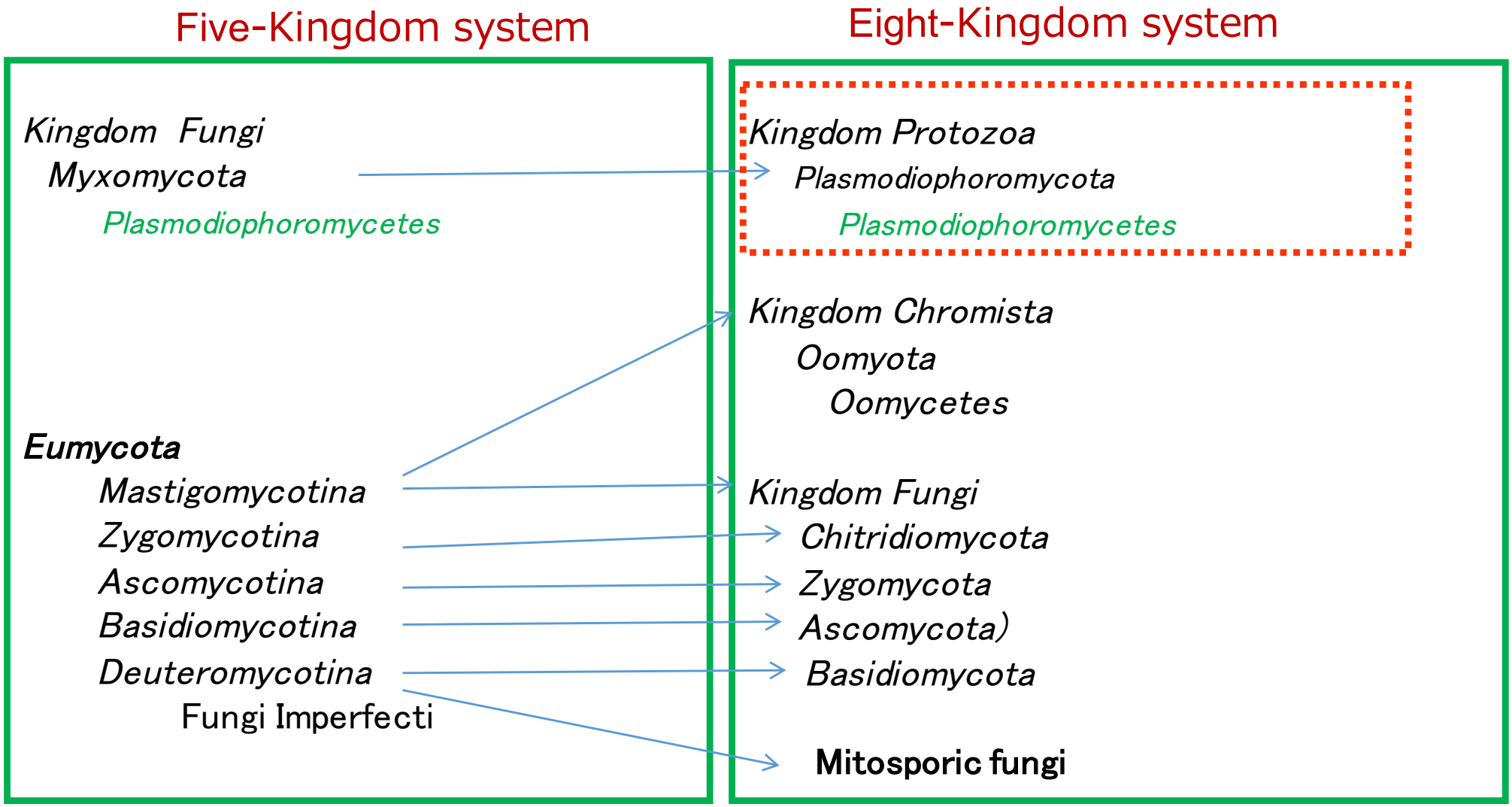
Clubroot is the most trouble soil pests

What kind of soil pests are you having trouble now?

From Japan agriculture newspaper (2007)



Taxonomic location of Clubroot



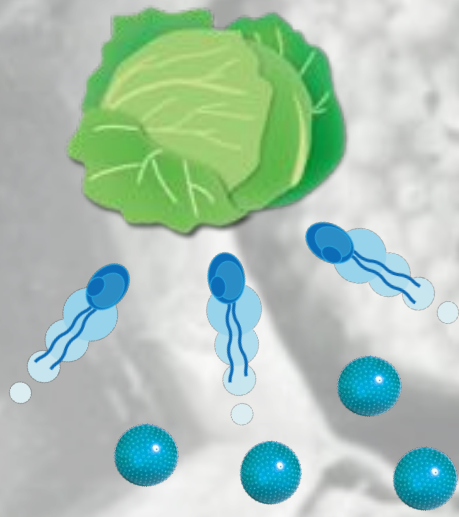
Not a fellow of fungus, but a fellow of protozoa, there are a few registered fungicides

Magar Fungicide against Clubroot in Japan

Common Name (registrated year)	Commercial Name	Target Site (FRAC CODE)	Clubroot life cycle	Shipment amount in 2016(¥M)
calcium cyanamide (1957～)	Sekkai-chisso	Improvement soil pH(-)		4,616
fluazinam (1990～)	Fronside	uncoupler of oxidative phosphorylation (29)	Inhibit resting spore germination	1,116
flusulfamide (1992～、2010～)	Nebijin	unknown (36)		1,050
amisulbrom (2010～)	Oracle	complex III: ubiquinone reductase at Qi site (21)	Killing Only Zoospore	1,090

More than **$30M fungicides** were used for Clubroot control.
Together with soil fumigant and pH improvement, over **$150M** is used.
Bait crops(Japanese leaf radish, oats, barley) are used for decrease resting spores.

Outline



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Measurement of clubroot resting spores in soil by LAMP* method

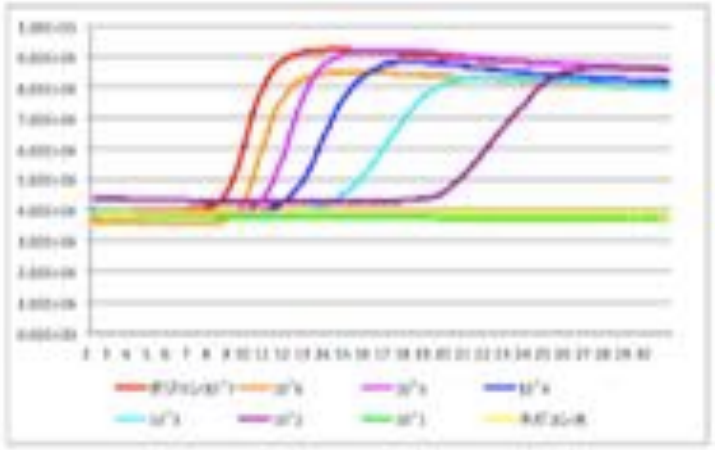
LAMP:Loop-Mediated Isothermal Amplification
LAMP reaction was carried out at 65 ℃. for 20 minutes for DNA Detection of clubroot.

＊：Gene amplification method developed by Eiken Chemical

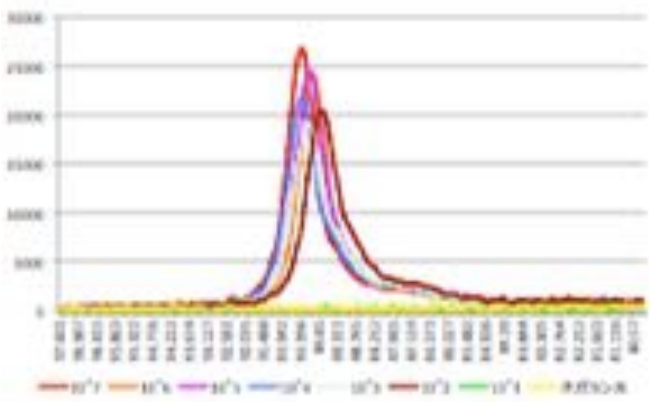
Simple and rapid gene amplification technology

Widely used for detection of infectious agent in medical field

The visual detection
Real Time PCR Detection
System or Genie®II



Amplification result of
LAMP method



Result of association
polarity analysis

Advantages of Improved LAMP method

		LAMP Standard method	LAMP Improvement method
Sample		Soil (0.4g)	Soil (0.4g)
DNA extraction		Benzyl chloride treatment	–
		Physically destroy (Beating with glass beads)	–
		Heating	Heating with Buffer (original methods)
		Cooling & Centrifugation	Centrifugation
		Purification by Magnetism Silica	Dilution of supernatant
Sensitivity (spores/g soil)		>1,000	>1,000 (commercial answer)
Time	DNA extraction	>3hr	20min
	Detection	15min	15min
	Total	**>4～5hr**	**<1hr**

Benefits of the LAMP method

Loop-mediated Isothermal Amplification (LAMP)

① Easy Handling

→ Reduction of Labor and Time

- Amplification of DNA at a constant temp.(65℃)

② No use Special Equipment

→ Advanced Research laboratory is not necessary

- Use easy equipment(constant temp. water tank, electric pots and jars)
- It comes to practical use and the assessment of numerous samples

③ No use high purification

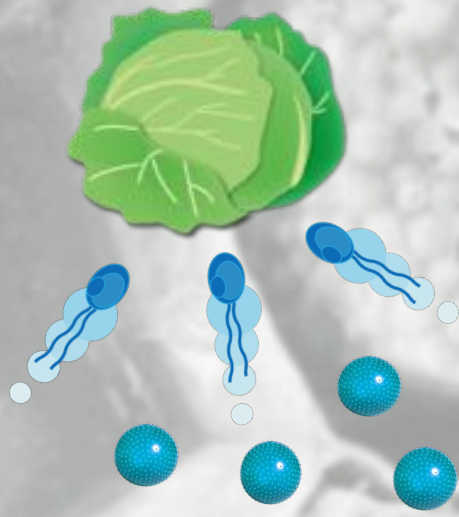
→ Small influence of reaction inhibitor

- DNA amplification reaction is possible even if template purification degree is low

• Possibility to make easy diagnosis in the field

- A kit can be made from DNA extraction to detection by LAMP
- Visual detection can be done negative or positive

Outline



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Clubroot Density Measurement Service

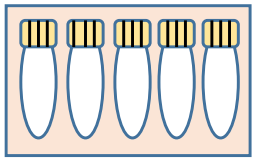
Order

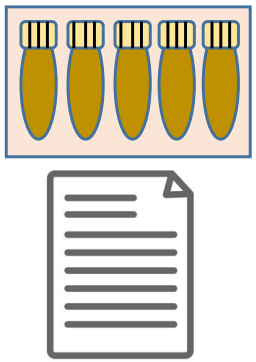
Sending of
the sampling kit
(collection tube)

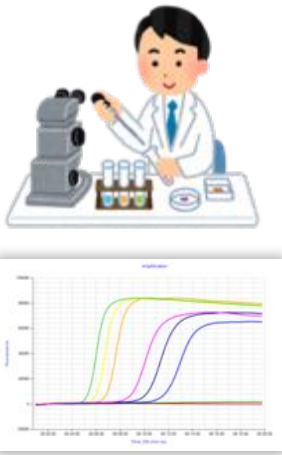
Sampling in field
Return of the soil
Clubroot history

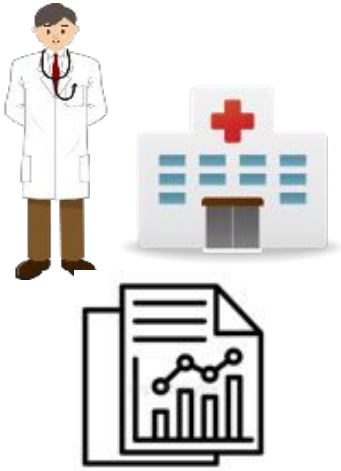
LAMP testing
(NIPPON
GENE)

Consulting
(vegetalia)









Reply within 1 week
(Shortest delivery time 3 days)

Analysis Data
Prescription

Diagnostic service price list

Launched from march/2017

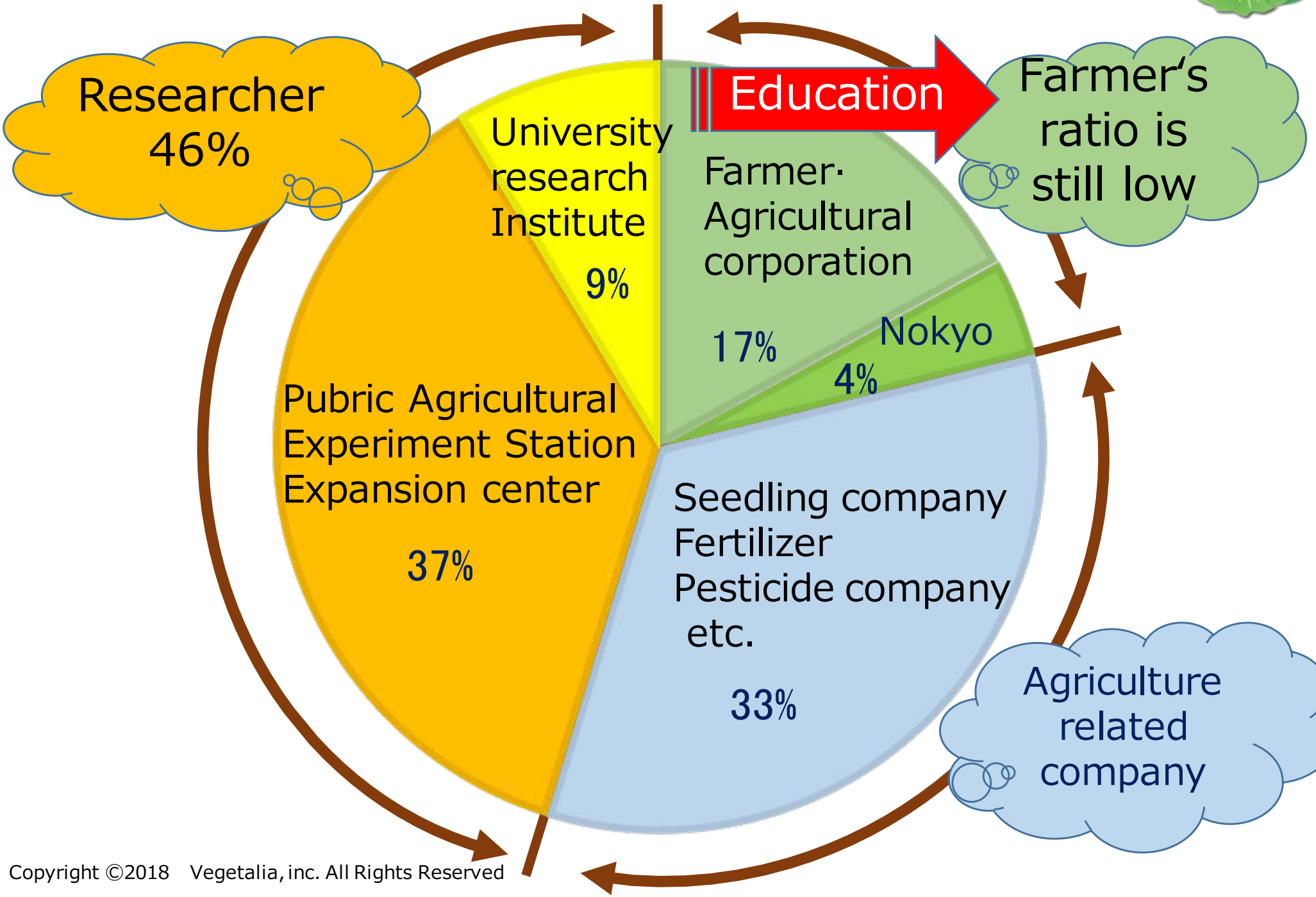
Including the charge for sending and returning the set

	Price	Cost per soil sample	Share
1sample	¥12,500+GST	$125	0.1%
5 samples set	¥20,000+GST	$40	**2.6%**
10 samples set	¥35.000+GST	$35	**97.3%**

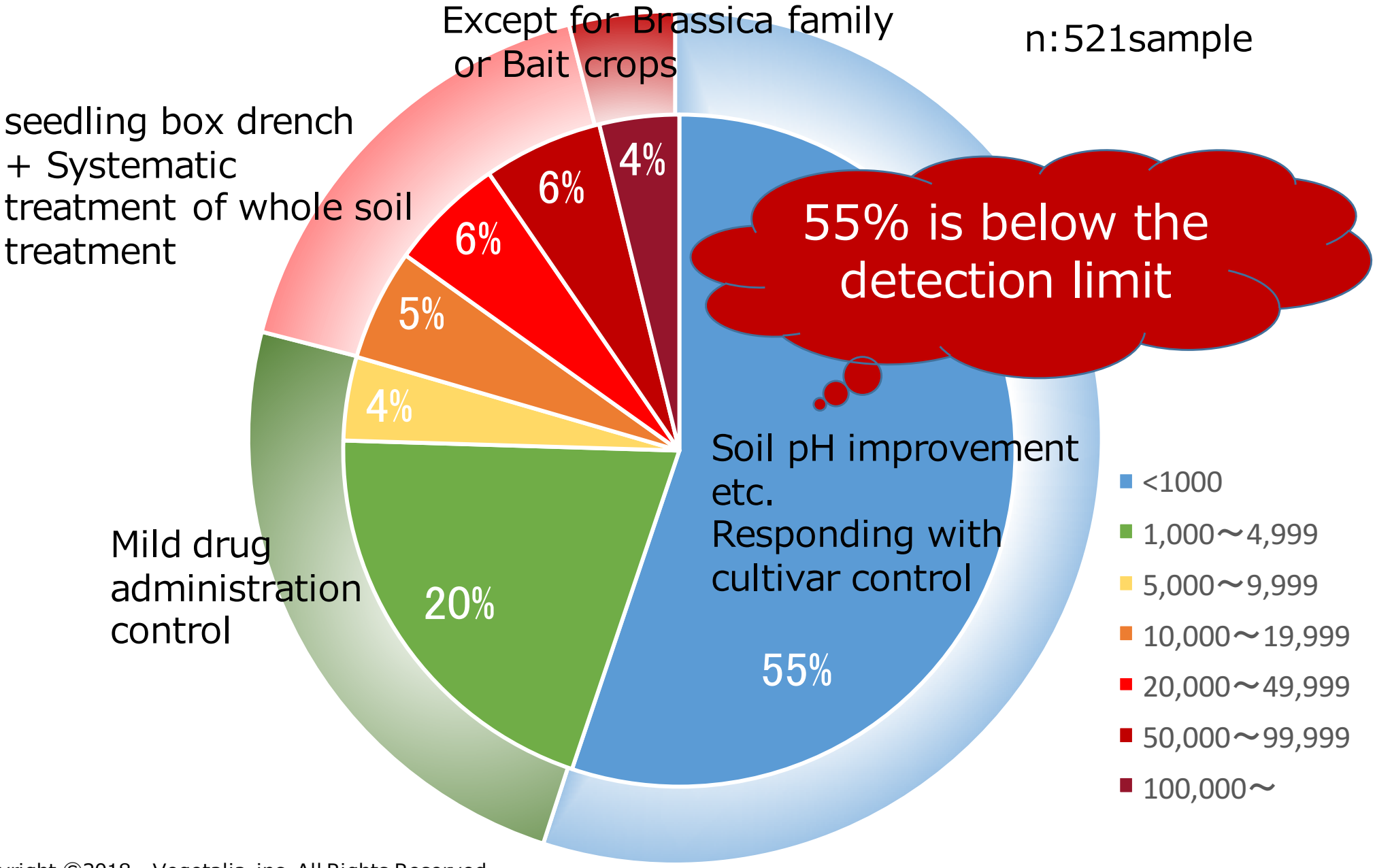
Cost is very important for social implementation

Examined more than 1,000 samples in the first year

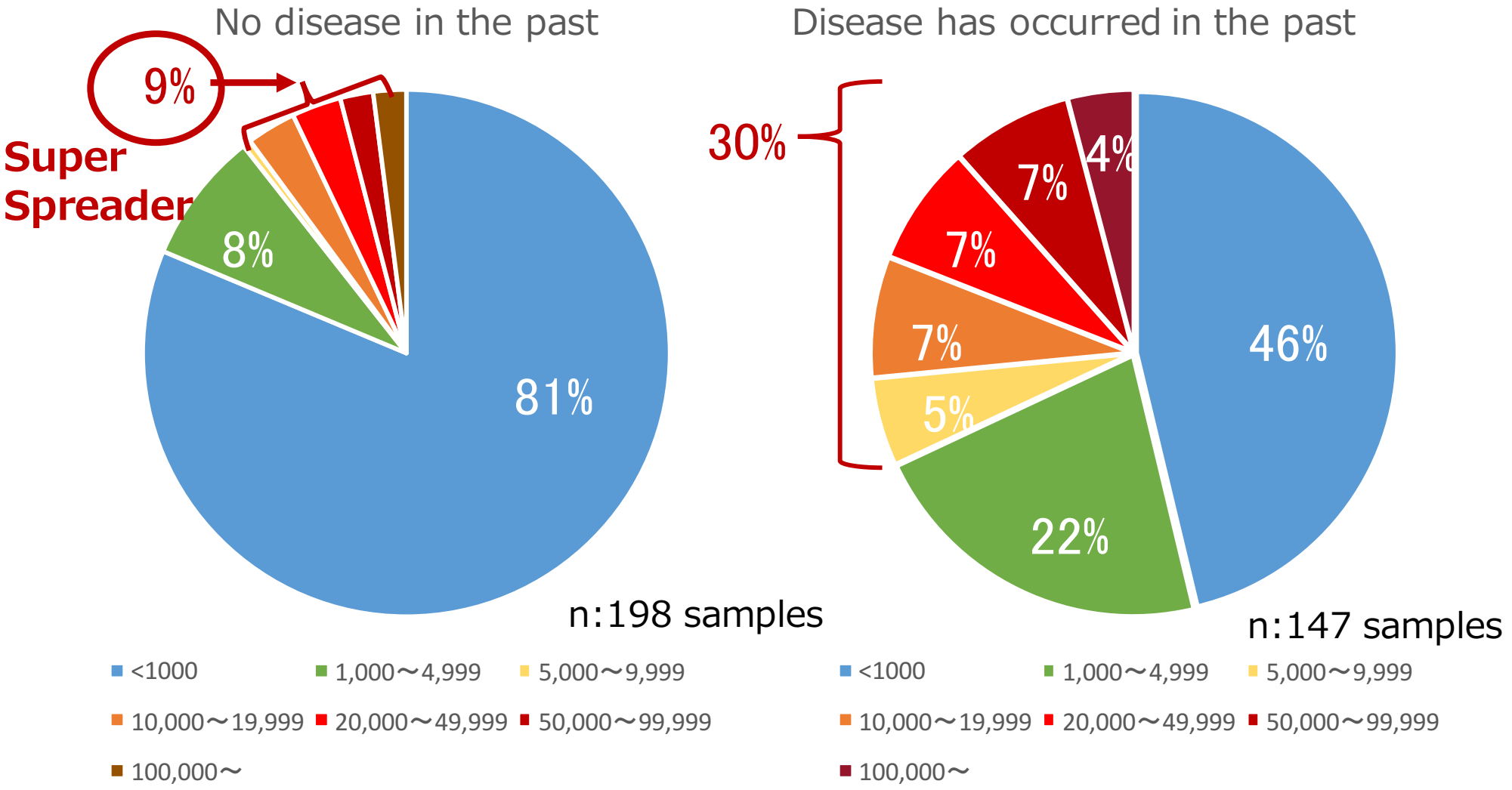
Client Categorization of Diagnostic service



Resting spore measurement and counter-measure



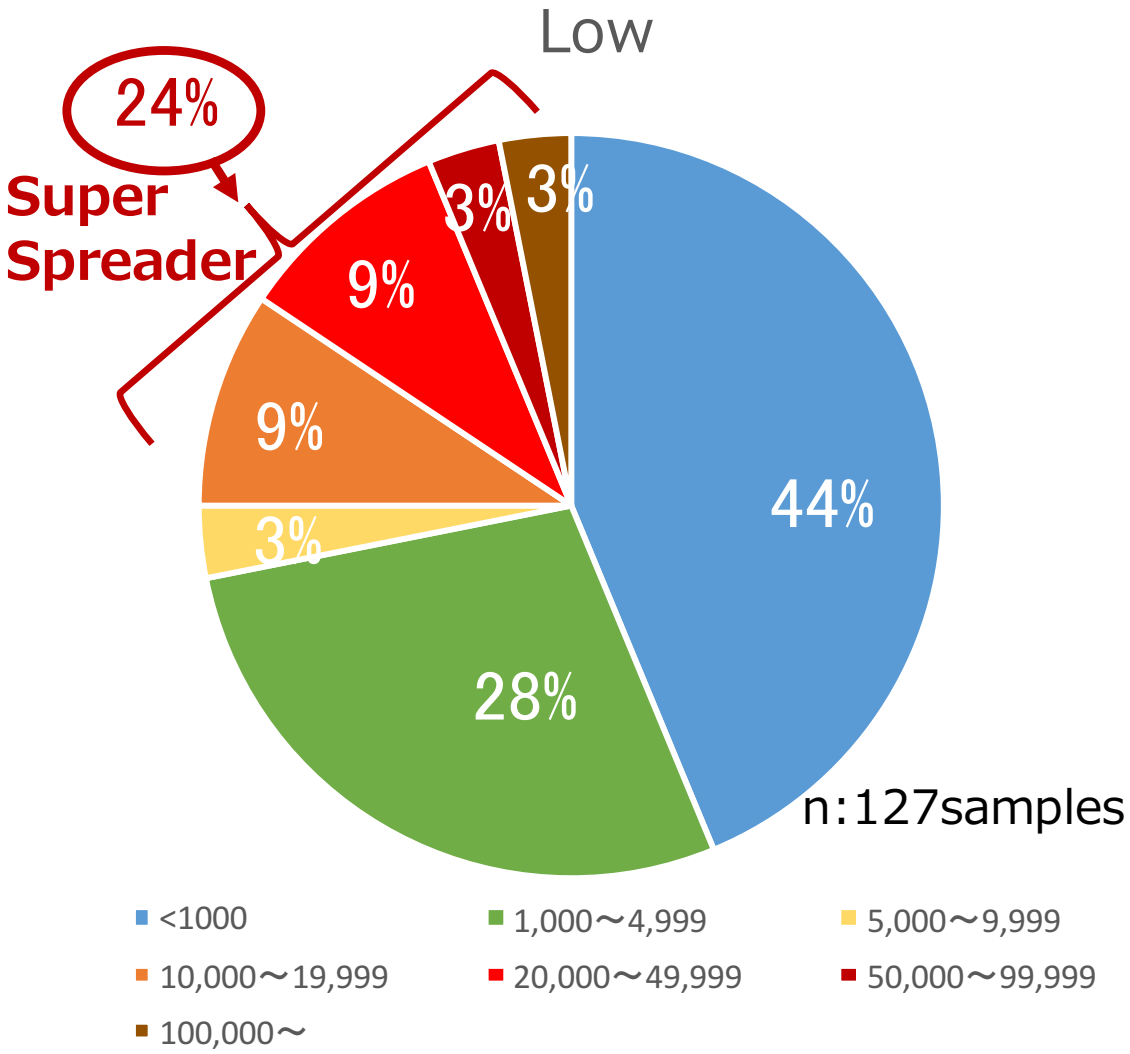
Correlation between disease history and resting spore contamination

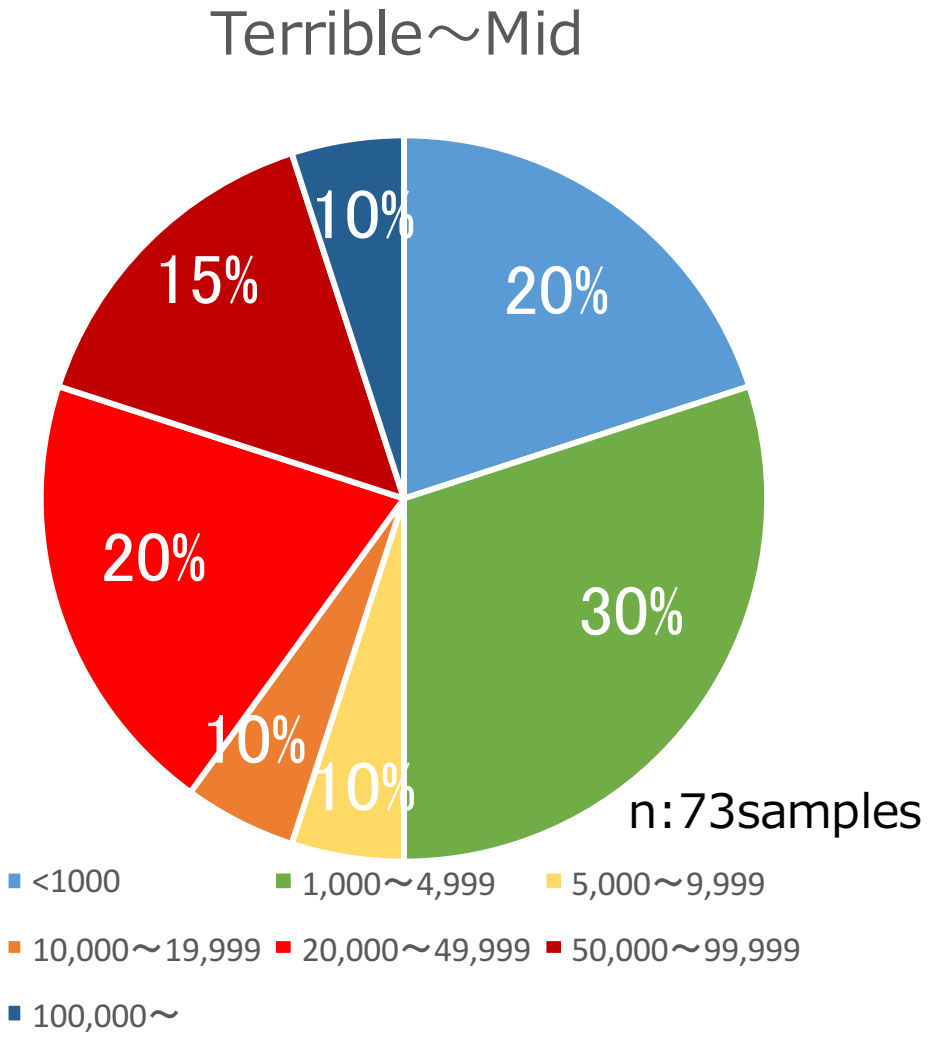


Even if there is no disease history
It may be a highly contaminated field

If there is disease in the past,
highly probable contaminated field

Relation between disease history and resting spores





Even if the degree of disease in the past is low, It may be a highly contaminated

If the degree of disease in the past is high, It may be a highly contaminated

(Example) Cabbage 1ha (10aX10fields)

Since the onset potential is unknown, apply fungicide in all

¥18,300 unknown	¥18,300 unknown	¥18,300 unknown	¥18,300 unknown	¥18,300 unknown
¥18,300 unknown	¥18,300 unknown	¥18,300 unknown	¥18,300 unknown	¥18,300 unknown

＜ Fungicide cost＞
10 fieldX@¥18,300
＝183,000（A）

Proper choice of controlling method from disease onset

¥18,300g 88,000 spores/g Consider crop changes	**¥18,300** 36,000 spores/g	¥3,300 6,000 spores/g	¥3,300 2,000 spores/g	¥0 Not detected
¥3,300 2,000 spores/g	¥3,300 4,000 spores/g	¥3,300 2,000 spores/g	¥3,300 1,,000 spores/g	¥3,300 1,000 spores/g

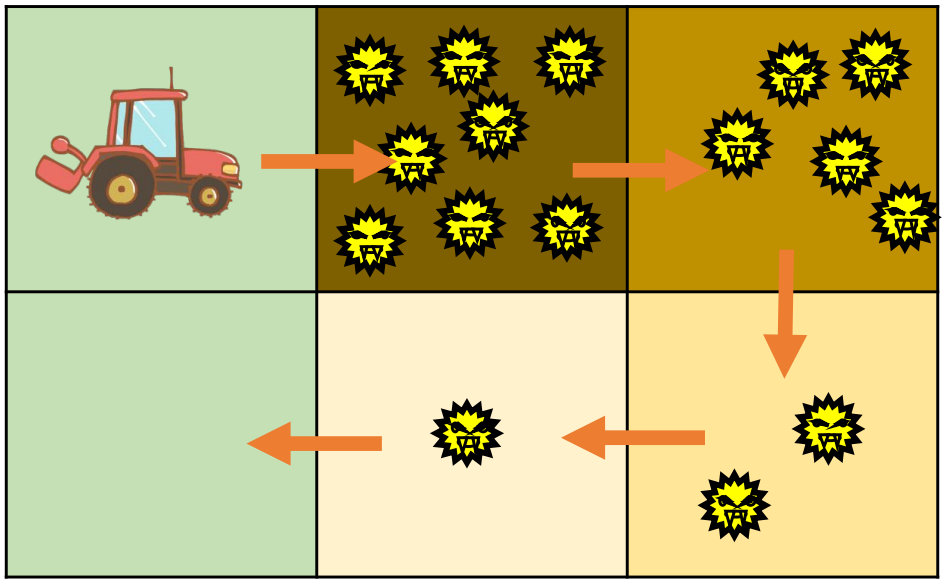
＜Diagnosis cost＞
=¥35,000
＜Fungicide cost＞
2 fieldsX@¥18,300
＝¥36,600
7 fieldsX@3,300
=¥23,100
Total **¥76,400 (B)**

It is possible to reduce the fungicide and work

A-B=¥106,600
Control cost cut 58%

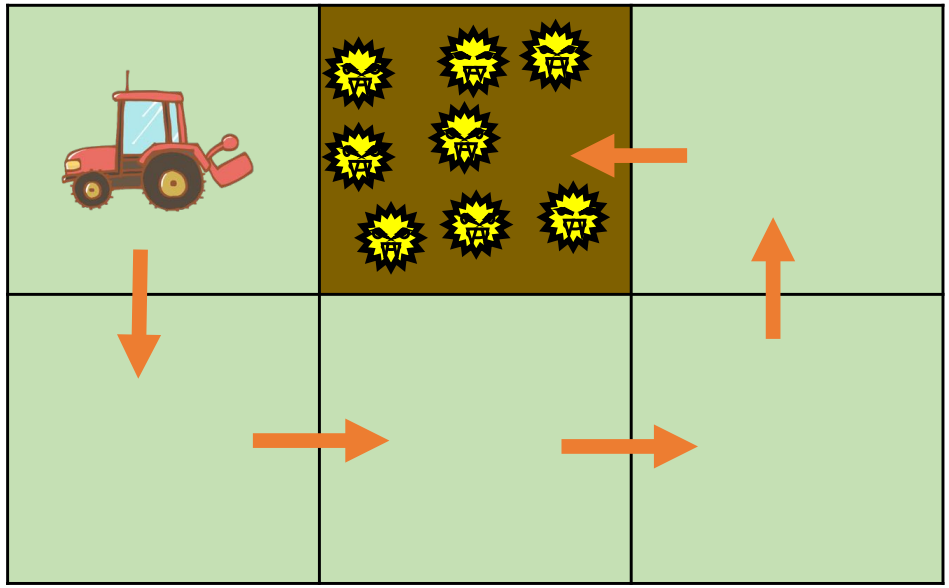
Knowing contamination of clubroot beforehand Control of district spreading

Work without knowing the contamination of clubroot



The pathogen through the field by the tractor

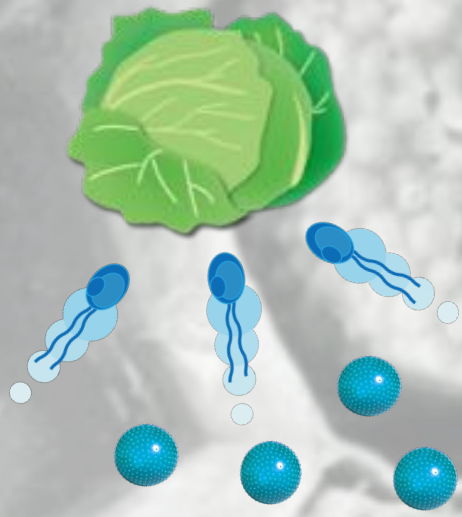
Investigate contamination in advance



Thorough control of contaminated fields, Fungicide cost reduction

- **Large scale farmer (broccoli about 500ha)**
  Possibility of reducing control cost about 50% down (¥20M➡¥10M)

Outline



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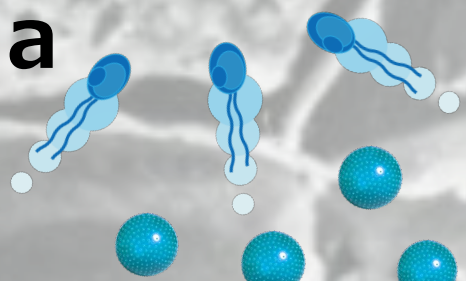
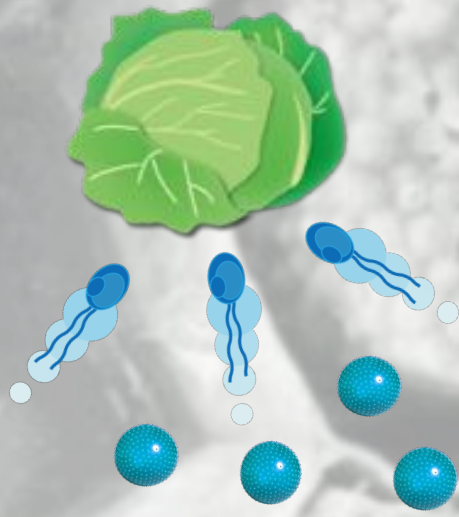
Measurement of clubroot resting spores in soil by LAMP method

- The LAMP method is effective for resting spore measurement in soil
- The only company in the licensed LAMP method patent
- Advantage in handling, measuring time, compared to standard method
- Looking for LAMP business partners all over the world, especially Canada





Thank you for your attention!

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