

## Biological Control of Root Diseases with dsRNA Based on Population Structure of Pathogens

Naoyuki MATSUMOTO

Department of Environmental Biology, National Institute of Agro-Environmental Sciences  
(Tsukuba, Ibaraki, 305 Japan)

### Abstract

Genetic individuals can be distinguished based on vegetative incompatibility in some fungi and are referred to as “genet”. They compete for resources with each other, and competition may result in disease suppression or decline, leaving a few fit genets. Double-stranded (ds) RNA of hypovirus origin, which infects healthy isolates through cytoplasmic contact and attenuates virulence, could promote decline where the population of pathogen is composed of a very few predominant genets. Previous studies on chestnut blight demonstrated that the spread of dsRNA was negatively correlated with the diversity in vegetative incompatibility in the population of the pathogen, *Cryphonectria parasitica*. I considered that finding applies to some diseases which remain difficult to control chemically, culturally, and, of course, biologically. *Helocobasidium mompa* and *Rosellinia necatrix*, the root rot pathogens of perennial crops, may be biocontrolled by dsRNA due to their simple population structure, their mode of epidemiology, and the high value of their individual host plants. Collaborative studies by concerned scientists are necessary to identify such viruses and to develop effective inoculation methods of indigenous genets with hypoviruses.

**Discipline:** Plant disease

**Additional key words:** hypovirulence, individualistic fungi, soilborne disease

### Introduction

The number of studies on biological control of soilborne plant diseases in a strict sense has decreased without an assessment of the cause of failure under field conditions<sup>2,4)</sup>. The failure is ascribed to the lack of ecological considerations on target diseases before the application of biocontrol. Antagonism produced in culture can seldom be reproduced in nature. Pathogen-antagonist interactions may be “thinned” in the complex ecosystem in soil. Both organisms should be close in niche to avoid thinning of interactions. Otherwise, specific antagonists must be selected for. In this paper, I review the possibility of biocontrol of soilborne diseases of perennial crops with double-stranded (ds) RNA which originates from the hypovirus. The hypovirus was known to infect through cytoplasmic contact, attenuating the virulence of many fungal pathogens (hypovirulence) and has been used for the biocontrol of chestnut blight under field conditions<sup>1,13)</sup>. Studies on population biology of the pathogen are a prerequisite for the effective spread of dsRNA<sup>25)</sup>.

### Genet: the lowest taxonomic unit

In the case of fungi it has been considered that individual genotypes were indistinguishable, mingling with each other in nature. This accepted theory does not apply to some fungi which have a self-nonsel self-recognition system based on vegetative (somatic) compatibility system. They are referred to as “individualistic” fungi<sup>40)</sup>. *Rhizoctonia solani* is the best-known of individualistic fungi among plant pathogens; strains belonging to the same anastomosis groups show the killing reaction immediately after hyphal anastomosis when they are different genetically<sup>30)</sup>. Rayner<sup>32)</sup> referred to such genotypes as genets. The term “genet” refers to genetic individuals originating from zygotes and was used in plant ecology<sup>12)</sup>. When a seedling is propagated vegetatively into more than 2 individual plants, they belong to a single genet even though they are separate physically. Monoovular twins are considered to be a single genet, as well.

Decayed stumps provide a good opportunity to observe genets of wood-decaying fungi<sup>40)</sup>. Each of the fruit bodies of *Coriolus versicolor* originates

from individual genets which occupy their own domain, excluding others in a stump. Demarcations between genets in the wood do not undergo a degradation process after mycelial interaction, resulting in hyphal cell death. Patches on turf caused by *Typhula ishikariensis* are another example of the domain of individual genets<sup>23</sup>. Fairy rings of *Marasmius oreades* indicate the marginal growth of genets<sup>4</sup>.

### Antagonism between genets

Individualistic fungi antagonize each other in the periphery of their domain so far as resources are available or the other party exists. Relationships between genets are dynamic, and the population structure tends to be simplified with fit genets selected for as a result of mutual antagonism<sup>23</sup>. Inoculation with several different strains of such fungi results in a decrease in disease severity as compared to inoculation with a single strain<sup>2,22</sup>. This phenomenon was attributed to a low inoculum potential after overall antagonism between strains, leading to the death of hyphae<sup>22</sup>. Though such experiments can not be easily conducted under field conditions, Smith<sup>36</sup> exploited this mechanism to control *Marasmius oreades* fairy rings on turf by thorough tillage of infested soil before replanting.

Root rot of sugarbeet caused by *Rhizoctonia solani* AG2-2 is known to decline after continuous monoculture<sup>15</sup>. There are many genets in a field, resulting in a complex population structure<sup>27,28</sup>. However, mutual antagonism could not be responsible for the decline phenomenon since, unlike *M. oreades*, *R. solani* AG2-2 produces basidiospores that are effective as inoculum<sup>26</sup> and consequently the field is replenished with new genets. Simplification of the pathogen may still occur after continuous monoculture because AG2-2 became predominant over several AGs of *R. solani* within a period of 5 to 6 years when disease decline became evident<sup>15</sup>.

### Decline of soilborne diseases and involvement of dsRNA

Root rot of sugarbeet caused by *R. solani* and take-all of wheat caused by *Gaeumannomyces graminis* var. *tritici* fail to develop after repeated monoculture, and this phenomenon is referred to as

“decline”<sup>18,35</sup>. Microorganisms are considered to be involved in the decline, but the causal microorganisms have not been identified so far.

Hyakumachi & Ui<sup>16</sup> reproduced the decline of sugarbeet root rot in pot experiments with 2 soils (Kiyokawa and Kitami soils) and 2 strains of *R. solani* AG2-2 (Rh-65 originating from Kiyokawa soil and H-17 from a site different from Kiyokawa and Kitami). Disease severity decreased considerably after the second seeding in all the pots containing Kiyokawa soil + Rh-65, Kiyokawa soil + H-17, or Kitami soil + Rh-65. Debilitated isolates of AG2-2 were recovered after 3–5 seedings only in pots with Kiyokawa soil + Rh-65, both originating from the same site. These isolates anastomosed with RH-65 without killing reaction<sup>17</sup>; i.e. they were vegetatively compatible. These isolates were obtained from the soil around diseased sugarbeet roots but were not strongly virulent. They neither contained dsRNA of viral origin nor transmitted hypovirulence to normal strains. As was described earlier, decline occurred in pots with materials of different geographic origins<sup>16</sup>. These facts, along with other findings, led Hyakumachi et al.<sup>18</sup> to conclude that the phenomenon was due to the increase in nonspecific antagonists, especially *Bacillus* spp. Castanho et al.<sup>5</sup>\*, however, detected dsRNA from debilitated strains of *R. solani* AG1 and confirmed the presence of cytoplasmic translocation.

Virus-like particles (VLPs) were found in *G. graminis* var. *tritici* isolated from wheat fields showing take-all decline in France (cited by Rawlinson et al.<sup>31</sup>). These isolates appeared to be responsible for the decline since they grew irregularly and were not virulent. Rawlinson et al.<sup>31</sup> recovered VLPs from the take-all fungus in U.K. The detection frequency of VLPs increased with continuous monoculture (up to 9 years), and they were transferred to another strain through hyphal anastomosis. Only a few isolates contained VLPs from 10 or 12-year-old monoculture fields showing decline, however. The involvement of VLPs in take-all decline requires experimental verification<sup>14</sup>.

Reduction in virulence was not considered to be responsible for the decline due to the fact that there was no difference in virulence in the isolates from long- and short-term monoculture fields<sup>3,9</sup>. Disease suppression factors related to decline can (1) be destroyed by heat moisture treatment at 60°C for

\* The term “decline” was used in their paper, too; however it indicated degenerated growth of the pathogen but not disease decline. Such strains were obtained after repeated transfers.

30 min, (2) be transferred from field to field, and (3) reproduce<sup>8)</sup>. Fluorescent pseudomonads, especially, seem to play an important role in disease suppressiveness, contributing to take-all decline. Suppressiveness results from the harmonized activity of various soil microorganisms, and the limited reproduction of introduced fluorescent pseudomonads was ascribed to the lack of key component(s) essential for disease decline<sup>39)</sup>.

Thus, hypoviruses may not be the main cause determining disease decline. However, the decline phenomenon has not been reported in other major soilborne diseases caused by non-individualistic fungi such as *Fusarium*, *Phytophthora*, *Pythium*, and *Verticillium*\*. Hypoviruses should prevail in the pathogen population just before the disease declines when the population structure of the pathogen becomes simple with a very few genets after mutual antagonism during continuous monoculture. Disease decline has not been investigated in terms of the change in population structure of the pathogen.

#### Chestnut blight: population structure and spread of dsRNA

Chestnut blight caused by *Cryphonectria parasitica* has been extensively studied in North America and Europe since dsRNA is considered to be a promising biocontrol agent, reducing the virulence of the pathogen. Investigations on population structure in the field are a prerequisite for the introduction of the agent. The virus is transmitted to healthy strains when field isolates and strains with the agent are vegetatively compatible; and, therefore, simple population structure is liable to the attack of dsRNA.

The disease was introduced in the early 1900s to the U.S.A.<sup>11)</sup> and to Europe<sup>13)</sup> and became epidemic. The trees which survived were not resistant, but the pathogen was found to be less aggressive. These strains were referred to as "hypovirulent" strains, and normal, virulent strains became hypovirulent when opposed to such strains in culture. Hypovirulent strains healed the damage when introduced to the periphery of canker. dsRNA was recovered from these strains, and subsequent experiments revealed that dsRNA was responsible for the hypovirulence and that it was of viral origin.

Hypoviruses infect fungi exclusively through

cytoplasmic contact between hyphae, and vegetative incompatibility is considered to be a mechanism for fungi to prevent infection<sup>6,40)</sup>. Consequently, the use of the viruses for biocontrol largely depends on the population structure of pathogens. Hypovirulence spreads fast when the pathogen population is composed of uniform genotypes. The extent of diversity in v-c (vegetative compatibility) groups in the chestnut blight fungus was correlated with the effectiveness of biocontrol with dsRNA. Greater success in Europe was ascribed to the lower diversity of the pathogen population as compared to that in North America<sup>13)</sup>. Chen et al.<sup>7)</sup> obtained transformant strains of *C. nectria* by introducing cDNA of the virus in order to overcome the defense barrier of the pathogen by vegetative incompatibility.

#### Prospect for exploitation of dsRNA in Japan

*Helicobasidium mompa* and *Resellinia necatrix* cause violet and white root rot in various crops, respectively. Fruit trees suffer from these diseases seriously in Japan; e.g. 400,000 (8.6%) apple trees were found to be affected by the disease in the 1978–1980 survey conducted in Aomori<sup>10)</sup>. These diseases can be controlled chemically for only a limited period of time<sup>41)</sup>. Biological control with *Trichoderma* spp. or actinomycetes was found to be ineffective under field conditions<sup>20)</sup>. Long-term control may be hampered by the perennial growth of hosts and by the difficulty in the development of antagonists in the rhizosphere. Short-term crop rotation is not applicable, either. The introduction of dwarf stock to apple orchards stimulates the occurrence of violet root rot<sup>33)</sup>.

An investigation on the population structure of *H. mompa* in an apple orchard using dwarf stock revealed that the population of the fungus was very simple with a huge, predominant genet, accounting for 82% of a total of 68 isolates<sup>19)</sup>. There were 8 other satellite genets consisting of up to 3 isolates. A large genet was found in 2 patches, and each isolate belonging to it seemed to be connected with each other in each patch. Since *H. mompa* on asparagus spreads by the extension of roots<sup>38)</sup>, it may infect other plants by root contact.

Although soil amendments are essential for the survival and control of root-infecting fungi<sup>29)</sup>,

\* Diseases may be suppressed by mycoparasites<sup>21)</sup> or by the increase in the incidence of nonpathogenic strains<sup>37)</sup>, but these examples do not fit to decline in a strict sense which occurs after continuous monoculture of susceptible crops. The word "decline" had been used to indicate a decrease in inoculum potential before the term was defined<sup>34)</sup>.

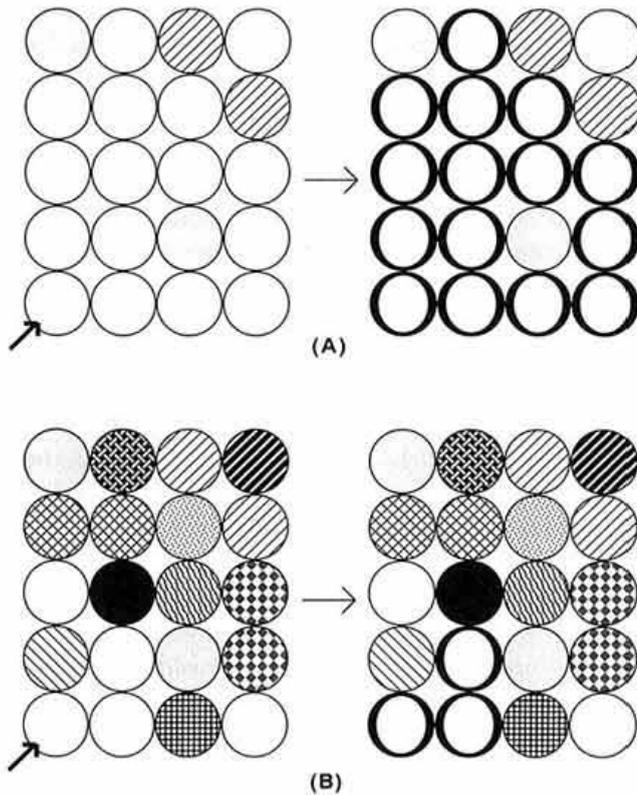


Fig. 1. Schematic representation of the mode of spread of hypovirulence in fields with simple (A) and diverse genets (B) of a pathogen

Where population structure is simple, hypovirulence spreads faster and the disease becomes attenuated more quickly. Each circle indicates the range of mycelia, and circles with the same patterns show the same genets. Circles with thick outer lines indicate attenuated individuals. Thick arrows show the spots of introduction of hypovirulence.

perennial growth habit of fruit tree roots makes it difficult to apply amendments deep in the soil. Hypovirus occurs within the cytoplasm of fungal hyphae, and no further management is necessary to promote its activity. It should spread in the network of fungal mycelia more effectively within orchards with simple population structure of the pathogen than in those with diverse genets (Fig 1).

## Conclusion

Few biocontrol experiments have been successful under field conditions in the control of soilborne diseases. Antagonists need to be in close contact with pathogens ecologically or physically. The hypovirus typically represents the latter since it occurs within the cytoplasm of the pathogen. The

biocontrol agent is least likely to be affected by the soil environment. Soil amendments with organic matter, etc. to help antagonists become established are not necessary in terms of disease control. Fruit trees are highly profitable on a plant basis, and biocontrol with dsRNA should be beneficial and, moreover, does not induce environmental pollution. Research collaboration is essential for the identification of the hypovirus, development of inoculation methods, and the selection of good viral strains. Also, knowledge on population biology of pathogens is important for understanding how dsRNA spreads in populations, as was emphasized in chestnut blight.

## References

- 1) Anagnostakis, S. (1982): Biological control of chestnut blight. *Science*, **215**, 466–471.
- 2) Årsvoll, K. (1976): Mutual antagonism between isolates of *Typhula ishikariensis* and *Typhula incarnata*. *Meld. Norg. LandbrHøgsk.*, **55**(19), 1–6.
- 3) Asher, M. J. C. (1980): Variation in pathogenicity and cultural characters in *Gaeumannomyces graminis* var. *tritici*. *Trans. Br. Mycol. Soc.*, **75**, 213–220.
- 4) Burnett, J. H. & Evans, E. J. (1966): Genetic homology and the stability of the mating-type factors of 'fairy rings' of *Marasmius oreades*. *Nature*, **210**, 1368–1369.
- 5) Castanho, B., Butler, E. E. & Shepherd, R. J. (1978): The association of double-stranded RNA with Rhizoctonia decline. *Phytopathology*, **68**, 1515–1519.
- 6) Caten, C. E. (1972): Vegetative incompatibility and cytoplasmic infection in fungi. *J. Gen. Microbiol.*, **72**, 221–229.
- 7) Chen, B. et al. (1996): Phenotypic changes associated with wild-type and mutant hypovirus RNA transfection of plant pathogenic fungi phylogenetically related to *Cryphonectria parasitica*. *Phytopathology*, **86**, 301–310.
- 8) Cook, R. J. (1990): Twenty-five years of progress towards biological control. In *Biological control of soil-borne plant pathogens*. ed. Hornby, D., CAB, Wallingford, 1–14.
- 9) Cook, R. J. & Naiki, T. (1982): Virulence of *Gaeumannomyces graminis* var. *tritici* from fields under short-term and long-term wheat cultivation in the Pacific Northwest. *Plant Pathology*, **31**, 201–207.
- 10) Fukushima, C. (1987): Recent state of violet root rot of apple trees in the Aomori Prefecture with some notes on the feature of their occurrence. *Shokubutsu-boueki*, **41**, 93–97 [In Japanese].
- 11) Fulbright, D. W., Paul, C. P. & Garrod, S. W. (1988): Hypovirulence: a natural control of chestnut blight. In *Biocontrol of plant diseases II*. ed. Mukerji, K. G. & Garg, K. L., CRC Press, Boca Raton, 121–139.
- 12) Harper, J. L. (1977): Population biology of plants. Academic Press, New York, pp. 892.

- 13) Heiniger, U. & Rigling, D. (1994): Biological control of chestnut blight in Europe. *Annu. Rev. Phytopathol.*, **32**, 581–599.
- 14) Hornby, D. (1979): Take-all decline: a theorist's paradise. In *Soil-borne plant pathogens*. ed. Schippers, B. & Gams, W., Academic Press, London, 133–156.
- 15) Hyakumachi, M. & Ui, T. (1982): Decline of *Rhizoctonia* root rot during sugarbeet monoculture. *Mem. Fac. Agric. Hokkaido Univ.*, **13**, 445–454. [In Japanese with English summary].
- 16) Hyakumachi, M. & Ui, T. (1982): Decline of damping-off of sugarbeet seedlings caused by *Rhizoctonia solani* AG2-2. *Ann. Phytopath. Soc. Jpn.*, **48**, 600–606 [In Japanese with English summary].
- 17) Hyakumachi, M. & Ui, T. (1984): Properties of diseased isolates of *Rhizoctonia solani* isolated from soil. *Ann. Phytopath. Soc. Jpn.*, **50**, 255–262 [In Japanese with English summary].
- 18) Hyakumachi, M., Kanzawa, K. & Ui, T. (1990): *Rhizoctonia* root rot decline in sugarbeet monoculture. In *Biological control of soil-borne plant pathogens*. ed. Hornby, D., CAB, Wallingford, 227–247.
- 19) Katsumata, H., Ogata, T. & Matsumoto, N. (1996): Population structure of *Helicobasidium mompa* in an apple orchard in Fukushima. *Ann. Phytopath. Soc. Jpn.*, **62**, 490–491.
- 20) Kojima, S. (1987): Control of white root rot in mulberry field. *Shokubutsuboueki*, **41**, 107–111 [In Japanese].
- 21) Lifshitz, R., Sneh, B. & Baker, R. (1984): Soil suppressiveness to a plant pathogenic *Pythium* species. *Phytopathology*, **74**, 1054–1061.
- 22) Matsumoto, N. & Tajimi, A. (1983): Intra- and intertaxon interactions among dikaryons of *Typhula incarnata* and *T. ishikariensis* biotypes A, B, and C. *Trans. Mycol. Soc. Jpn.*, **24**, 459–465.
- 23) Matsumoto, N. & Tajimi, A. (1993): Effect of cropping history on the population structure of *Typhula incarnata* and *Typhula ishikariensis*. *Can. J. Bot.*, **71**, 1434–1440.
- 24) Matsumoto, N. (1994): Report on biocontrol research presented at 6th ICPP (1) Biocontrol of fungal diseases with antagonists. *PSJ Biocont. Rep.*, **4**, 24–27.
- 25) Milgroom, M. G. (1994): Population biology of the chestnut blight fungus, *Cryphonectria parasitica*. *Can. J. Bot.*, **73**(suppl.1), S311–S319.
- 26) Naito, S. & Sugimoto, T. (1980): Relationship between basidiospore dispersal of *Thanatephorus cucumeris* (Frank) Donk and development of foliage blight of sugar beets. *Ann. Phytopath. Soc. Jpn.*, **46**, 216–223 [In Japanese with English summary].
- 27) Nishimura, N. & Sugimoto, T. (1985): Anastomosis-compatibility of *Rhizoctonia solani* AG2-2. *Res. Bull. Hokkaido Natl. Agric. Exp. Stn.*, **142**, 1–7 [In Japanese with English summary].
- 28) Ogoshi, A. & Ui, T. (1983): Diversity of clones within an anastomosis group of *Rhizoctonia solani* Kühn in a field. *Ann. Phytopath. Soc. Jpn.*, **49**, 239–245 [In Japanese with English summary].
- 29) Papavizas, G. C. (1975): Residues and amendments in relation to survival and control of root-infecting fungi: an introduction. In *Biology and control of soil-borne plant pathogens*. ed. Bruehl, G. W., American Phytopathological Society, St. Paul, 76.
- 30) Parmeter, J. R., Sherwood, R. T. & Platt, W. D. (1969): Anastomosis grouping among isolates of *Thanatephorus cucumeris*. *Phytopathology*, **59**, 1270–1278.
- 31) Rawlinson, C. J. et al. (1973): Virus-like particles in the take-all fungus, *Gaeumannomyces graminis*. *Ann. Appl. Biol.*, **74**, 197–209.
- 32) Rayner, A. D. M. (1991): The phytopathological significance of mycelial individualism. *Annu. Rev. Phytopathol.*, **29**, 305–323.
- 33) Sakuma, T. (1987): The latest trend and further prospect of research on violet and white root rot of fruit trees. *Shokubutsuboueki*, **41**, 91–92 [In Japanese].
- 34) Semeniuk, G. & Henry, A. W. (1960): Relative decline of *Ophiobolus graminis*, *Helminthosporium sativum* and *Fusarium culmorum* in the soil. *Can. J. Plant Sci.*, **40**, 288–294.
- 35) Shipton, P. J. (1975): Take-all decline during cereal monoculture. In *Biology and control of soil-borne plant pathogens*. ed. Bruehl, G. W., American Phytopathological Society, St. Paul, 137–144.
- 36) Smith, J. D. (1980): Is biologic control of *Marasmius oreades* fairy rings possible? *Plant Disease*, **64**, 348–354.
- 37) Sneh, B., Pozniak, D. & Salomon, D. (1987): Soil suppressiveness to *Fusarium* wilt of melon, induced by repeated croppings of resistant varieties of melons. *J. Phytopathology*, **120**, 347–354.
- 38) Suzui, T. (1978): Studies on the ecology and the control of asparagus violet root rot caused by *Helicobasidium mompa* Tanaka. *Res. Bull. Hokkaido Natl. Agric. Exp. Stn.*, **122**, 87–165 [In Japanese with English summary].
- 39) Thomashow, L. & Weller, D. M. (1990): Application of fluorescent pseudomonads to control root diseases of wheat and some mechanisms of disease suppression. In *Biological control of soil-borne plant pathogens*. ed. Hornby, D., CAB, Wallingford, 109–122.
- 40) Todd, N. K. & Rayner, A. D. M. (1980): Fungal individualism. *Sci. Prog. Oxf.*, **66**, 331–354.
- 41) Umemoto, S. (1987): Control of white root rot of Japanese pear with isoprothiolane. *Shokubutsuboueki*, **41**, 102–106 [In Japanese].

(Received for publication, April 1, 1997)