論文の内容の要旨

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論文題目 Effects of reproductive interference between *Bursaphelenchus xylophilus* and *B. mucronatus* on the development of pine wilt disease
（マツノザイセンチュウとニセマツノザイセンチュウの繁殖干渉がマツ材線虫病の発病に及ぼす影響）

Pine wilt disease is an infectious disease of pine trees. It is caused by the nematode *Bursaphelenchus xylophilus* (Steiner et Buhrer) Nickle and is transmitted by cerambycid beetles of the genus *Monochamus*. The pathogenic nematode native to North America has been introduced to East Asia and westernmost Europe, where the non-pathogenic, closely-related nematode *B. mucronatus* is distributed widely. Once *B. xylophilus* invades susceptible pine forests, *B. mucronatus* is displaced rapidly by *B. xylophilus*. The species displacement has been implicitly considered to result from the interspecific competition for limited resources.

Reproductive interference is caused by incomplete recognition of the other species and reduced fitness by mating attempts and copulation with the other species and so on. Interspecific competition through reproductive interference is characterized by asymmetric, frequency-dependent competition and brings several ecological and evolutionary consequences on species. *Bursaphelenchus xylophilus* and *B. mucronatus* are known to produce $F_1$ hybrids that cannot establish populations in most cases, suggesting the presence of reproductive interference between the two species. The aim of this study was to determine the effects of interspecific competition of nematode species on the development of pine wilt disease and the characteristics of their competition.

**Suppressive effects of *B. mucronatus* on pine wilt disease development and *B. xylophilus* populations in pine seedlings**

Theories predict that one of the two competing species populations quickly displaces the other through reproductive interference in a frequency-dependent manner. Thus it is anticipated

that *B. mucronatus* suppresses the virulence of *B. xylophilus* against pine trees when *B. mucronatus* inoculated heavily outnumber *B. xylophilus*.

To determine the suppressive effects of *B. mucronatus*, 15,000 nematodes including an isolate of *B. xylophilus*, T4, and an isolate of *B. mucronatus*, Srf, whose hybrids break down, were inoculated on each of 30 3-year-old *Pinus thunbergii* seedlings at three combinations of different numbers, and *B. xylophilus* alone was inoculated on 30 other seedlings in early August. Inoculation of *B. mucronatus* significantly retarded the speed of foliage discoloration and significantly prolonged the survival time of seedlings. *Bursaphelenchus mucronatus* significantly reduced the nematode density per gram of dried seedling stem. Analysis of rDNA genotypes showed 1846 *B xylophilus*, no *B. mucronatus* and one hybrid.

To determine when the interspecific competition occurred in the former experiment, I inoculated 4,000 T4 nematodes, 11,000 Srf nematodes, or 15,000 nematodes of mingled isolates on 3-year-old *P. thunbergii* seedlings in late July and sampled them in 4, 8, 12, and 16 weeks after the inoculation. Genotyping of rDNA showed extremely small proportions of *B. mucronatus* and hybrids in Week 4 and their disappearance in Week 8, indicating that the interspecific competition almost finished within four weeks of the inoculation. However, the reproductive interference from *B. mucronatus* influenced negatively the population size of *B. xylophilus* even after its disappearance, i.e. the mean nematode densities in seedlings were smaller when the two species were inoculated simultaneously than when *B. xylophilus* alone.

**Interspecific competition between *B. xylophilus* and *B. mucronatus***

Interspecific competition between animal species occurs through exploitation and interference including biological conditioning. Especially, reproductive interference is caused by incomplete recognition of heterospecific opposite sex and the production of inviable and sterile hybrids. Theoretically, one species population takes longer to displace the other species population by the exploitative competition than the competition through reproductive interference, in which species displacement occurs in frequency-dependent manner. Using isolates of the two *Bursaphelenchus* species whose hybrid populations do not persist, I investigated the relationship between the outcomes of competition and their population traits. Values of intrinsic rate of natural increase, *r*, and carrying capacity, *K*, were estimated to be 0.854/ day and 72,361, 0.509/ day and 28,272, and 1.10/ day and 120,062 for isolates T4, Srf, and TBm119, respectively on *Botrytis cinerea* fungal mat in 50-mm-diameter Petri dishes at 25 °C. When 50 nematodes were placed on the *B. cinerea* fungal mat at seven different ratios of isolates T4 and Srf of 10:0, 9:1, 7:3,

5:5, 3:7, 1:9 and 0:10, molecular techniques showed the occurrence of the hybrids in a week. Those also revealed that T4 displaced Srf in 1-2 and 2-4 weeks when being inoculated at proportions of 0.9 and 0.7, respectively, whereas Srf displaced T4 in two of the three dishes in 4 and 4-5 weeks when being inoculated at proportions of 0.9 and 0.7, respectively. On the other hand, T4 displaced another *B. mucronatus* isolate TBm119 in 1-2 weeks when being inoculated at a proportion of 0.9, while TBm119 displaced T4 in 2-4, 1-4, and 1-2 weeks when being inoculated at proportions of 0.5, 0.7, and 0.9.

Quick, frequency-dependent species displacement between *B. xylophilus* and *B. mucronatus* were characteristics of interspecific competition through reproductive interference. Based on mathematical models of reproductive interference and resource competition by Kuno (1992), zero-growth isoclines were depicted using estimates of *b*, *d*, *h*, *c*, by the experimental results. Comparing the observations and the predictions indicated the interspecific competition through reproductive interference rather than competition for resources. Difference in species displacement patterns between two *B. mucronatus* isolates was explained in part by smaller *r* and *K* values in Srf than in T4 and by larger *r* and *K* values in TBm119 than in T4.

**Interspecific copulation between *B. xylophilus* and *B. mucronatus***

To determine the recognition ability between conspecific and heterospecific opposite sexes in copulation, a virgin adult of one sex and two virgin adults of the opposite sex of two nematode species were placed on PAD medium containing *Nectria viridescens*. Genotyping of rDNA of F1 offspring showed the difference in sex-specific recognition ability between isolates. Females of the two species had higher abilities of species recognition than the males; males copulated with heterospecific opposite sex more frequently than the females in the present of those of the same species. In the absence of conspecific males, *B. mucronatus* females accepted *B. xylophilus* males more frequently than *B. xylophilus* females accepted the heterospecific males. Especially, Srf females (15/ 16) more often accepted T4 males in copulation compared with TBm119 females (18/ 22). On the other hand, there was no difference in copulation rate with the heterospecific females between the males of the two species in the absence of the conspecific females.

**Frequency-dependent inhibitory effects of *B. mucronatus* on the number of *B. xylophilus* carried by *Monochamus alternatus***

The number of *B. xylophilus* carried by newly-emerged *Monochamus alternatus* adults, the initial nematode load, determines the transmission ability of nematodes; even one beetle with

heavy nematode loads can transmit an enough number of nematodes to induce pine wilt disease. To determine the effects of *B. mucronatus* on the boarding of *B. xylophilus* onto *Monochamus alternatus* beetles, beetle larvae were singly into artificial holes of pine bolts together with 2,000 nematodes at seven different ratios of the two nematode species. Mean initial nematode loads were smaller when bolts were inoculated with the two species than when with *B. xylophilus* alone, indicating the inhibition of *B. xylophilus* boarding by *B. mucronatus*. Proportions of *B. xylophilus* in the nematode loads were more than 0.8 when the percentage of *B. xylophilus* in the inoculum was 50 % or more, whereas those were less than 0.1 when 30 % or less. That was caused by the frequency-dependent inhibition of *B. xylophilus* population growth by *B. mucronatus* in pine bolts and higher boarding abilities of *B. xylophilus*. However, higher boarding rates of *B. xylophilus* in small populations in xylem than in huge population may explain the difficulty of exclusion of *B. xylophilus* in natural conditions.

**General discussion**

This thesis showed that interspecific competition between *B. xylophilus* and *B. mucronatus* occurs through reproductive interference when their hybrids cannot establish a population. Laboratory experiments using the two nematode species revealed quick species displacement and frequency dependency, which are characteristics of competition through reproductive interference. Frequency-dependent competition patterns differed depending on nematode isolates used. That was explained in part by differences in the intrinsic rate of natural increase, carrying capacity, and incomplete recognition of heterospecific opposite sex. Interspecific crossing studies so far conducted show the hybrid breakdown in most cases. The experiments showed that this type of competition affected the development of pine wilt disease and the initial nematode load of insect vectors in frequency-dependent manner. Thus, competition through reproductive interference is considered to be responsible in part for the displacement of *B. mucronatus* by *B. xylophilus* observed in the field.