Host Status of Three Transgenic Plum Lines to Mesocriconema xenoplax

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Abstract. The expression of gastrodianin antifungal protein (GAFP) in a form of its VNF isoform increases tolerance to Phytophthora root rot (Phytophthora cinnamomi) and the root-knot nematode (Meloidogyne incognita) in transgenic plum lines. However, nothing is known about the potential of the GAFP lectin to confer disease resistance to the ring nematode, Mesocriconema xenoplax, in plum. Three transgenic plum lines (41, 4J, and 5D) expressing gafpl under the control of CaMV 35S promoter sequence were evaluated for their response to M. xenoplax in the greenhouse. All plum lines were rated as hosts of M. xenoplax. Among the individual plum lines tested, the number of M. xenoplax per gram of dry roots was lowest in the rhizosphere of transgenic line SD, intermediate in that of the nontransformed control line, and greatest in line 4J. The results of this study indicate that the comparisons of the final soil densities (Pf) of adult and juvenile M. xenoplax expressed as nematodes per gram of dry roots provide a better measure of the nematode carrying capacity by the tested lines than Pf values referred to as number of M. xenoplax/100 cm$^3$ soil.

In the southeastern United States, the productive lifespan of peach [Prunus persica (L.) Batsch] trees does not exceed 6 to 10 years on some sites as a result of premature tree mortality (Brittain and Miller, 1978). Two causes of early tree death are a disease complex known as peach tree short life (PTSL) and 'Armillaria' root rot (Miller, 1994; Savage and Cowart, 1942). Peach tree short life is reportedly caused by a predisposition of trees to cold injury, bacterial canker (Pseudomonas syringae pv. syringae van Hall), or a combination of both, which results from feeding by the ring nematode, Mesocriconema xenoplax (Raski; 1952) Loôf and de Grisse, 1967 (Meloidogyne incognita) in transgenic plum lines. However, nothing is known about the potential of the GAFP lectin to confer disease resistance to the ring nematode, Mesocriconema xenoplax, in plum. Three transgenic plum lines (41, 4J, and 5D) expressing gafpl under the control of CaMV 35S promoter sequence were evaluated for their response to M. xenoplax in the greenhouse. All plum lines were rated as hosts of M. xenoplax. Among the individual plum lines tested, the number of M. xenoplax per gram of dry roots was lowest in the rhizosphere of transgenic line SD, intermediate in that of the nontransformed control line, and greatest in line 4J. The results of this study indicate that the comparisons of the final soil densities (Pf) of adult and juvenile M. xenoplax expressed as nematodes per gram of dry roots provide a better measure of the nematode carrying capacity by the tested lines than Pf values referred to as number of M. xenoplax/100 cm$^3$ soil.

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et al. (2008). Agrobacterium tumefaciens-mediated transformation resulted in three gfp-1 expressing plum lines, which were designated 41, 4J, and 5D. Three transgenic and nontransformed plum lines were evaluated in two greenhouse tests. Plum lines were clonally propagated from the original, nonhost (highly resistant), Rf = 0; poor host (resistant), Rf = 0.01 to 0.99; and good host (susceptible), Rf 1 or greater. The test was repeated once. In the second test, younger (63-d-old) transgenic and a nontransformed plum line along with 11-d-old Nemaguard peach seedlings were inoculated 6 d after transplanting and exposed to the nematode infection for 181 d after inoculation (i.e., 22 May 2008 to 19 Nov. 2008). Inoculation procedures, PI, seedling handling in the greenhouse, and parameters recorded were the same as those of the previous test.

Nematode data were $\log_{10} (x + 1)$ transformed and subjected to analysis of variance with the general linear models procedure of SAS (SAS Institute, Cary, NC). Appropriate preplanned single-degree-of-freedom comparisons were then used to detect differences between treatment means for Nemaguard peach versus combined plum line means following a significance F. Means within plum lines were analyzed using Tukey's honestly significant difference test. Actual numerical data were used for table presentation. Only significant differences ($P \leq 0.05$) are discussed unless stated otherwise.

### Results and Discussion

All plum lines combined supported greater ($P \leq 0.05$) numbers of *M. xenoplax* than Nemaguard peach (known susceptible) in Test 1. A similar trend occurred in Test 2 although differences were not significant (Table 1). However, when the final nematode population density was expressed on a per gram of dry root basis, no differences were detected between the combined plum lines and Nemaguard in both tests, indicating that all plum lines combined supported similar nematode populations as Nemaguard. Root dry mass and nematode infestation levels as measured by number of *M. xenoplax* mobile life stages per gram of dry root is a better measure of host resistance/susceptibility than nematodes per 100 cm$^3$ soil, because it standardizes the nematode populations among the different plant species tested based on total root mass. Using this criterion has proven a useful tool in the preliminary identification of tolerance in Guardian® to *M. xenoplax* (Nyczepir et al., 1996). It was determined that specific Guardian® lines suppressed *M. xenoplax* populations relative to Nemaguard rootstock, but not Lovell. Among the plum lines tested, the number of *M. xenoplax* per gram of dry root was lowest ($P \leq 0.05$) with transgenic line 5D, intermediate with the nontransformed control line, and greatest with line 41 in both tests. In Test 2, transgenic line 41 also supported a greater ($P \leq 0.05$) number of *M. xenoplax* per gram of dry root than line 5D, and in Test 1, a similar trend was detected although differences were not significant. The lower final nematode densities observed on the transgenic plum line 5D reflect a more vigorous and developed root system of this line compared with the other lines tested in this study and also that of Nemaguard peach rootstock. This observation is substantiated in that total dry root weight for transgenic line 5D (Tests 1 and 2 = 12.11 and 22.51 g, respectively) was greater than transgenic lines 41 (Tests 1 and 2 = 7.30 and 7.54 g, respectively) and 4J (Tests 1 and 2 = 9.69 and 8.65 g, respectively) and also the nontransformed control line (Tests 1 and 2 = 8.04 and 7.48 g, respectively) and Nemaguard peach (Tests 1 and 2 = 2.11 and 5.66 g, respectively) (data not presented in Table 1).

Plants with large root systems usually support larger nematode populations than plants with reduced root mass. It is not certain why transgenic line 5D, with a larger root system than the other transgenic lines, supported fewer *M. xenoplax* per gram of dry root, but this specific transgenic line is known to have different genetic and disease performance characteristics than transgenic lines 41 and 4J (Nagy et al., 2008).

For each experiment, line 5D had the lowest number of gfp-1 insertions (versus 41 = one copy and 4J = two copies). Despite these potential genetic advantages, line 5D is more susceptible to *Phytophthora cinnamomi* infection than transgenic lines 41 and 4J. Furthermore, transgenic lines 41, 4J, and 5D were all shown to support lower populations of the Southern root-knot nematode (*M. incognita*) compared with the inoculated control line, but greatest effects on suppression of root-knot nematode galling and reproduction were observed in transgenic lines 4J and 41. Two possible explanations for the different response of transgenic line 5D when exposed to the infestation of a species (*M. xenoplax*) belonging to another nematode genus having different parasitic habits may be attributed to 1) specific feeding sites on the root and nourishment needed to be exposed at the root surface and 2) multiple gfp-1 gene copies in this line 5D. Nematode feeding sites on roots differ between a sedentary endoparasite such as the root-knot nematode and a migratory endoparasite such as the root nematode. *Meloidogyne* spp. penetrate at the root tip, become sedentary within the root, and form feeding sites called giant cells within the vascular cylinder region. These endoparasites remain sedentary and feed on established giant cells for the remainder of their life cycle (de Guinan and Ritter, 1979).

In contrast, ring nematodes feed from individual cortical cells further back on the root for up to 8 d and then move to a new feeding site along the root (Hussey et al., 1992, which is modified into discrete food cells. In this study, transgenic line 5D appears to provide less nourishment to *M. xenoplax* than lines 4J and 41, which is contrary to its effect on *M. incognita* (Nagy et al., 2005). It is not certain if the GAFP lecin in transgenic plum line 5D suppressed *M. xenoplax* populations through feeding or direct contact, but like *M. incognita*, *M. xenoplax* requires specialized feeding cells for sustenance and reproduction.
Lectins are carbohydrate-binding proteins that have been found in many plants and their properties have been linked to a variety of plant functions, including defense against various plant pathogens (Hu et al., 1988; Koo et al., 2002; Lee et al., 2003; Van Damme et al., 1998; Wang et al., 2001, 2004). It was reported that expression of a monocot mannose-binding lectin (GNA) conferred partial resistance to M. incognita in Arabidopsis (Ripoll et al., 2003). The mechanism of plant resistance is not known, but it is believed that GNA may bind glycoproteins on nematode-associated with amphids and (or) the nematode surface. Such disruption would ultimately interfere with nematode sensory discernment and the ability of the nematode to form the essential feeding cells needed for nourishment (Thomas and Cottage, 2005).

Furthermore, it was reported that some transgenic Arabidopsis lines were more resistant to M. incognita than others and that the most resistant lines did not contain the most copies of the T-DNA insertion region containing the GNA-expression cassette (Ripoll et al., 2003). A similar phenomenon was reported when gafp-1-expressing plum lines were challenged with M. incognita (i.e., transformed lines 4J and 4I, but not line 5D) (Nagel et al., 2008). In contrast, transgenic plum lines 4J (one gafp-1 gene copy) and 4I (two gafp-1 gene copies) supported greater M. xenoplax populations than line 5D (four gafp-1 gene copies) when compared on a per gram of dry root basis. It appears that increased copy number or transcript expression levels may be correlated with suppression of M. xenoplax populations, but not M. incognita.

All plum lines tested in this study were rated as susceptible hosts (Rf 0-1) compared on a per number of gafp-1 gene copies) when compared on a gram of dry root basis. It appears that increased copy number or transcript expression levels may be correlated with suppression of M. xenoplax populations, but not M. incognita.

### Table 1: Population density of *Mesocrictonema xenoplax* on plum (*Prunus domestica* cv. Stanley) lines and peach cultivars in the greenhouse after 180 d.

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*RF = reproductive factor (P/P), where P = final population density of Al. xenoplax juveniles and adults/100 cm² soil and P = initial population density of 10 M. xenoplax juveniles or adults/100 cm² soil. RF rating, as follows: nonhost (highly resistant), RF = 0; poor host (resistant), RF = 0-0.99; and good host (susceptible), RF 1 or greater.

*a Data are means of 10 replicates. 
*b Means within plum lines and column followed by the same letter are not different (P ≤ 0.05).

The single-degree-of-freedom comparison between the means for peach versus combined plum lines was significant (P ≤ 0.05).

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