Effects of *Ph$_p$* Gene-Associated versus Induced Resistance to Tobacco Cyst Nematode in Flue-Cured Tobacco

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Abstract: Effects of the systemic acquired resistance (SAR)-inducing compound acibenzolar-*S*-methyl (ASM) and the plant-growth promoting rhizobacterial mixture *Bacillus subtilis* A13 and *B. amyloliquefaciens* IN937a (GB99+GB122) were assessed on the reproduction of a tobacco cyst nematode (TCN- *Globodera tabacum solanacearum*) under greenhouse conditions. Two sets of two independent experiments were conducted, each involving soil or root sampling. Soil sample experiments included flue-cured tobacco cultivars with (*Ph$_p$*+: NC71 and NC102) and without (*Ph$_p$*-: K326 and K346) a gene (*Ph$_p$*) suppressing TCN parasitism. Root sample experiments examined TCN root parasitism of NC71 and K326. Cultivars possessing the *Ph$_p$* gene (*Ph$_p$*+) were compared with *Ph$_p$*- cultivars to assess the effects of resistance mediated via *Ph$_p$* gene vs. induced resistance to TCN. GB99+GB122 consistently reduced nematode reproductive ratio on both *Ph$_p$*+ and *Ph$_p$*- cultivars, but similar effects of ASM across *Ph$_p$*- cultivars were less consistent. In addition, ASM application resulted in leaf yellowing and reduced root weight. GB99+GB122 consistently reduced nematode development in roots of both *Ph$_p$*+ and *Ph$_p$*- cultivars, while similar effects of ASM were frequently less consistent. The results of this study indicate that GB99+GB122 consistently reduced TCN reproduction in all flue-cured tobacco cultivars tested, while the effects of ASM were only consistent in *Ph$_p$*+ cultivars. Under most circumstances, GB99+GB122 suppressed nematode reproduction more consistently than ASM compared to the untreated control.

Key words: biological control, management, induced resistance.

During the course of plant-pathogen interactions, plants protect themselves against pathogens in several ways. According to Huang (1998), plant defense mechanisms can be either preformed (passive or basal) or induced (active). Preformed resistance is constitutively present in plants and expressed constantly. In contrast, induced resistance occurs when a plant recognizes invading pathogens through various elicitors and initiates defense reactions against them (Zacheo et al., 1997). Exogenous application of certain biotic agents or chemical compounds analogous to pathogen elicitors can also trigger induced resistance in plants, which are categorized either as systemic acquired resistance, SAR, or induced systemic resistance, ISR (Pieterse and van Loon, 2007; Dietrich et al., 2004). Mechanisms of induced resistance are assumed to improve plant disease protection and reduce costs associated with crop protection practices (Heidel and Dong, 2006).

Studies in the patterns of constitutive and induced resistance against insect herbivory demonstrate that plants modulate these two defense mechanisms in different plant parts (roots vs. leaves vs. fruit) based on the probability of attack (Zangerl and Rutledge, 1996). Therefore, constitutive or induced resistance may not be equally expressed throughout the plant. Optimization of defense reactions against pathogens comprising both constitutive and induced resistance components has not been well studied. In fact, the majority of the research to date has focused on comparing the effects of constitutive and induced resistance on insect herbivory (Adler and Karban,

1994; Åström and Lundberg, 1994; Karban and Myers, 1989; Padilla and Adolph, 1996; Parker, 1992; Rhoades, 1979; Tuomi et al., 1991). In order to maximize the chances of disease protection through resistance mechanisms under field conditions, it is necessary to study how these two types of resistances interact with each other. Thus, comparing the effects of induced resistance with and without constitutive resistance against a particular pathogen may provide additional information, such as any synergistic or antagonistic effects of constitutive gene expression on induced resistance. Strong synergistic effects between these resistance mechanisms could provide significantly enhanced and stable resistance.

Tobacco cyst nematode (TCN), *Globodera tabacum solanacearum* (Miller & Gray, 1972) Behrens, 1975 is an important pathogen of flue-cured tobacco in Virginia (Johnson et al., 2009). Yield losses in flue-cured tobacco in Virginia have been estimated to average approximately 15%, with a few occurrences of complete crop failure (Crowder et al., 2003). Nematicide application is routine to management of TCN even when resistant cultivars are available to farmers. However, nematicides are environmentally unfriendly, expensive, and are highly regulated with increasingly restricted availability (Crowder et al., 2003; C. S. Johnson, VPI & SU, pers. com.).

Four *Nicotiana* spp.- *N. glutinosa* L., *N. paniculata* L., *N. plumbaginifolia* Viv., and *N. longiflora* Cav., were found to possess resistance to TCN in the early 1970s (Baalawy and Fox, 1971). The same research group demonstrated that hybrids developed from *N. plumbaginifolia* were highly resistant to TCN. Prior to 1996, researchers had very limited success in developing a TCN-resistant cultivar with yield and leaf quality equal to commonly grown susceptible cultivars. Only a few TCN-resistant cultivars were available, and the yield and leaf quality from these cultivars were lower than commonly planted TCN-susceptible cultivars (Hayes et al., 1997). Since 1996, hybridization has been used to develop flue-cured

tobacco cultivars heterozygous for a single, dominant gene (*Ph$_p$*) originally transferred from *N. plumbaginifolia* (Johnson et al., 2009). The *Ph$_p$* gene provides complete resistance to race 0 of the black shank pathogen (*Phytophthora nicotianae* (B. De Haan), and also significantly reduces TCN population densities (Johnson et al., 2009). These hybrid cultivars combine resistance to black shank and TCN with high yield and leaf quality characteristics (Johnson et al., 2009; Johnson et al., 2002; Crowder et al., 2003).

We demonstrated in earlier research that application of an SAR-inducing compound, acibenzolar-*S*-methyl (ASM), and a mixture of the PGPR *Bacillus subtilis* A13 and *B. amyloliquifaciens* IN937a [GB99+GB122; BIOYIELD, Bayer CropScience Research Triangle Park, NC)] suppressed TCN reproduction by an average of 60% in oriental (cv. Xanthi NN) and flue-cured tobacco (cv. K326) (Parkunan, 2008). In this study, our objectives were to compare the effects of ASM and GB99+GB122 on TCN parasitism of cultivars with and without *Ph$_p$* gene associated resistance to TCN.

Materials and Methods

Two sets of independent greenhouse experiments were conducted in both 2007 and 2008 at the Southern Piedmont Agricultural Research and Extension Center, Blackstone, VA, each set involving either soil or root sampling. Independent experiments were repeated once. The first set of greenhouse experiments examined TCN reproduction in soil, and was arranged in a split-plot design with 18 replications. Main plot treatments included an ASM foliar spray, incorporating GB99+GB122 into potting soil, and an untreated control. Subplot treatments randomized within each main plot treatment consisted of four flue-cured tobacco cultivars, two with the *Ph$_p$* gene (*Ph$_p$*+: NC71 and NC102) and two without (*Ph$_p$*-: K326 and K346). TCN reproduction is generally similar among flue-cured tobacco cultivars with or without the *Ph$_p$* gene (Johnson et al., 2009). The second set of greenhouse experiments was also arranged in a split-plot design, but included five replications and involved counting TCN numbers in tobacco roots to assess nematode penetration and development. Main plot treatments included were: ASM foliar spray, GB99+GB122-amended soil, or an untreated control. Subplot treatments again included TCN-susceptible (*Ph-*: K326) and resistant (*Ph+*: NC71) flue-cured tobacco cultivars.

Source and application methods of inducers: Combinations of GB99+GB122 obtained from Gustafson LLC (now Bayer CropScience, Research Triangle Park, NC) were used as an ISR inducing agent. The bacteria were formulated on a dry flake carrier for a bacterial concentration of 3×10^9 cfu/g. Approximately 0.18 g of the product, containing 5.3×10^8 cfu of bacteria, was added to 300 cm^3 of soil mixture to achieve a rate equivalent to 593.5 g of product per cubic meter of soil mixture. ASM (ACTIGARD 50WG, 50 % a.i., Syngenta Crop Protection, Greensboro, NC) was applied as a foliar spray, 200 mg product/liter (equivalent to the labeled rate of 34.6 g/ha), to run off, every 10 days, starting two days after transplanting and continuing for 10 weeks, for seven total sprays in the soil sample experiment. In contrast to the soil sample experiment, the ASM foliar spray was only applied once in the root sample experiment.

Seedling preparation and transplanting: Seeds of NC71, NC102, K326 and K346 were placed in a peat-based tobacco growth medium (Carolina Choice Tobacco Mix, Carolina Soil Company, Kinston, NC) in an aluminum germination pan. Unless otherwise stated, plants were fertilized with 150-200 ppm nitrogen using a liquid 16:5:16 NPK fertilizer (ULTRASOL, SQM North America, Atlanta, GA). Four weeks after seeding, individual seedlings of equal size were transplanted into a 2.5-cm perforated plastic tray containing 50-cells, and placed on fertilized bay water. One week later, equal size plants of all four cultivars were re-transplanted into 10-cm diam. clay pots for the soil sample experiment. Plants of cultivars NC71 and K326 were similarly re-transplanted into 7.0-cm diam. clay pots for the root sample experiment. Ten centimeter pots were filled prior to transplanting with a mixture of 200 cm^3 steam-sterilized topsoil and 100 cm^3 PROFILE porous ceramic, greens grade, soil conditioner (Profile Products LLC, Buffalo Grove, IL). Seven centimeter-diameter pots were filled prior to transplanting with a mixture of 66 cm^3 steam sterilized topsoil and 33 cm^3 PROFILE porous ceramic soil conditioner. GB99+GB122 was mixed separately into the topsoil:profile mixture prior to filling the appropriate pots. Soil in each pot was fertilized with 150 ml of water-soluble fertilizer every week. Plants were watered equally from above as needed to maintain satisfactory moisture.

Nematode inoculum preparation and infestation: TCN cultures were maintained continuously on tomato cv. Rutgers in the greenhouse. Cysts were extracted from the soil every ninety days and re-cultured onto a fresh tomato plant. Cysts were extracted using a modified Fenwick can (Caswell et al., 1985) and crushed in a blender for one minute to release eggs. Eggs were rinsed through a 150 μm sieve nested over a 25 μm sieve. Eggs retained on the 25 μm sieve were diluted to 500 eggs/ml with tap water. One week after final transplanting, or five days after ASM or GB99+GB122 treatment, 10 ml of the nematode egg suspension (approximately 5,000 eggs) for the soil sample experiment and 14 ml of egg suspension (approximately 7,000 eggs) for the root sample experiment were poured onto the soil surface in each pot and covered with 100 and 50 cm^3 of soil, respectively, for the soil and root sample experiments, and lightly watered from the top.

Nematode extraction from soil samples: Ten weeks after infestation, mature cysts were extracted from each pot of the soil sample experiment using a modified Fenwick can (Caswell et al., 1985) and subjected to sucrose

centrifugation (Byrd et al., 1976). Numbers of cysts were counted under x40 and crushed in a blender to release eggs, which were then counted after staining with acid fuchsin (Byrd et al., 1983). Numbers of stained eggs were counted under 40x after sub-sampling two 10 ml aliquots from a 250 ml suspension. Reproductive ratio was calculated by dividing the final egg population/ 400 cm^3 (P$_f$) by the initial egg inoculum level (P$_i$ = 5,000). Additionally, plant morphological characteristics such as number of chlorotic leaves, total number of leaves, fresh leaf and root weight, and dry root weight were measured at the end of each experiment. Percent leaf yellowing was calculated from number of chlorotic leaves and total number of leaves.

Root staining: The entire root system was removed three weeks after nematode infestation from each pot in the root sample experiments, washed gently to remove soil, and blotted dry. Feeder roots were cut into sections approximately 0.5-cm-long and soaked in 1.6 percent sodium hypochlorite for five minutes to surface sterilize the root pieces and to remove any soil adhering to the root surface. Root pieces were rinsed with tap water on a 25 µm sieve and transferred back to 100 ml of tap water. Ten milliliters of acid-fuchsin stain was added to the suspension of root sections and microwaved for 70-sec at the medium power level (Byrd et al., 1983). The stained roots were transferred to a 25 µm sieve, rinsed with tap water, and transferred back to the flask after draining. Finally, 50 ml of acidified glycerin was added to the root pieces, which were then microwaved for 70-sec at medium power. Stained TCN in the root sections were counted under a stereomicroscope at 40x and categorized as vermiform (second-stage juveniles that had successfully penetrated into the root, but weren't obviously feeding), swollen (distinct sausage-shaped stage juvenile), flask-shaped or pyriform, and saccate adult females bearing eggs. The number of vermiform juveniles found inside the root indicated the penetration level and the combined number of other stages indicated the nematode developmental level.

Statistical analysis: Analyses of variance (PROC GLM) were conducted using SAS 9.1v software (SAS Institute, Cary, NC) to evaluate treatment effects. Data were log-transformed ($\log_{10}X+1$) prior to statistical analysis. Treatment means were compared using the Waller-Duncan *t*-test (k-ratio =100).

RESULTS

Soil sample experiment: The GB99+GB122 treatment reduced eggs per gram dry root weight, final numbers of eggs per 400 cm^3 soil and nematode reproductive ratio (*k*-ratio = 100) on both *Ph$_p$*- and *Ph$_p$*+ cultivars (Table 1). In contrast, ASM reduced eggs per gram dry root weight, final numbers of eggs per 400 cm^3 soil and nematode reproductive ratio on *Ph$_p$*+ cultivars in both experiments, but on *Ph$_p$*- cultivars only in the spring 2008 test. Cysts per 400 cm^3 soil, cysts per gram dry root weight, and fecundity were lower (*k*-ratio =100) for GB99+GB122 compared to the untreated control on *Ph$_p$*+ and *Ph$_p$*- cultivars in both the fall 2007 and spring 2008 trials. However, ASM reduced the number of cysts per 400 cm^3 soil on both the *Ph$_p$*- and *Ph$_p$*+ cultivars only in the spring 2008 test, and did not reduce cysts per gram dry root weight in either test. Reduced fecundity was associated with ASM only for *Ph$_p$*+ cultivars, and only in the spring 2008 test (Table 1). Cyst size and shape for all three treatments on *Ph$_p$*+ and for untreated control on *Ph$_p$*- cultivars are indicated in Figures 1A and 1B, respectively. Reduced fecundity was also associated with GB99+GB122 on *Ph$_p$*- cultivars, but similar effects of ASM on *Ph$_p$*- cultivars were less consistent (Table 1).

Increased leaf yellowing was associated with the ASM treatment on both *Ph$_p$*+ and *Ph$_p$*- cultivars in both tests compared to the untreated control and GB99+GB122 treatments (Table 2). Percent leaf yellowing for GB99+GB122 was less than the untreated control in the fall 2007 test. No difference in fresh leaf weight due to ASM versus the untreated control was observed in the fall 2007 test, but GB99+GB122 lowered (*k*-ratio = 100) fresh leaf weight compared to ASM in the *Ph$_p$*- cultivars in that trial. ASM and GB99+GB122 increased fresh leaf weight in the *Ph$_p$*+ cultivars in the spring 2008 test.

TABLE 1. Effects of induced resistance elicitors on tobacco cyst nematode reproduction on flue-cured tobacco cultivars with and without the *Ph$_p$* gene in greenhouse tests conducted at the Southern Piedmont Agricultural Research and Extension Center, Blackstone, Va in fall 2007 and spring 2008.*

Cultivars	Treatment	Cysts/400 cm^3 soil		Eggs/400 cm^3 soil		Cysts/g dry root weight		Eggs/g dry root weight		Reproductive ratio (P$_f$/P$_i$)		Fecundity (Eggs/cyst)	
		2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
Ph$_p$+(NC71 and NC102)	GB99+GB122	0.5b	0.7c	14.6b	45.4c	0.2b	0.1b	6.3b	9.8c	0.004b	0.009b	10.3b	37.7c
	ASM	0.9ab	1.1b	23.3b	71.2b	0.4ab	0.4a	11.5b	25.5b	0.006b	0.014b	14.2ab	48.5b
	Untreated	1.3a	2.4a	78.8a	225.7a	0.6a	0.5a	37.3a	44.2a	0.019a	0.045a	39.1a	84.6a
Ph$_p$-(K326 and K346)	GB99+GB122	14.1b	6.1c	1128.5b	524.9c	6.1b	1.2b	487.6b	113.8c	0.282b	0.105c	70.9b	94.8b
	ASM	26.2a	9.8b	1913.2ab	1297.4b	11.2a	3.5a	775.0ab	488.4b	0.478ab	0.259b	77.3ab	124.7ab
	Untreated	23.9a	17.9a	2068.8a	2783.8a	9.5a	3.8a	824.0a	568.7a	0.517a	0.557a	87.4a	156.9a

* Data presented are non-transformed means of 36 observations across 18 replications and 2 cultivars. Data were log-transformed [Log$_{10}$(x+1)] prior to statistical analysis. Means within a column followed by the same letter are not significantly different according to the Waller-Duncan *t* test (*k*-ratio =100).
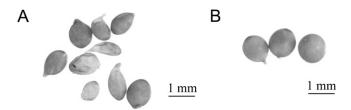
A **B**

1 mm 1 mm

FIG. 1. Cysts collected from resistant, Ph_p+ (A: NC71 and NC102 – from untreated control, acibenzolar-S-methyl and GB99+GB122 treatments) and susceptible, Ph_p- (B: K326 and K346 - untreated control) cultivars.

Fresh and dry root weight (k-ratio = 100) were lower for ASM in the spring 2008 test compared to both the untreated control and GB99+GB122 for both Ph_p- and Ph_p+ cultivars. However, fresh root weight was higher (k-ratio = 100) for the ASM treatment versus GB99+GB122 for Ph_p- cultivars in the fall 2007 test. Dry root weight for the ASM treatment was lower (k-ratio = 100) than GB99+GB122 for Ph_p+ cultivars in the fall 2007 test. Treatment with GB99+GB122 increased (k-ratio = 100) the fresh root weight of the Ph_p- cultivars compared to the untreated control, but only in the spring 2008 test (Table 2).

Root sample experiment: The trends among treatments in nematode numbers within a whole root and per gram root weight were similar for preparasitic (vermiform) and parasitic nematodes (Table 3). Fewer vermiform nematodes were present in GB99+GB122-treated NC71 and K326 compared to the untreated control in 2008, but similar trends in 2007 were not statistically significant (k-ratio = 100). Differences in numbers of vermiform TCN between ASM and the untreated control were never statistically significant. Both ASM and GB99+GB122 reduced the number of swollen TCN in K326 in both fall 2007 and spring 2008 tests. However, reduction of swollen TCN by ASM in NC71 was significant (k-ratio = 100) only in 2007, while reductions by GB99+GB122 were statistically significant in both tests. Pyriform juveniles were found only for the untreated control, but so few were found in TCN-resistant NC71 that differences between the ASM and GB99+GB122 treatments and the untreated control were only signif-

icant (k-ratio = 100) for TCN-susceptible K326. Adult were also only found in roots of K326 from untreated controls, but at very low numbers, so that treatment effects were not statistically significant (Table 3).

DISCUSSION

Results from these experiments confirm those from previous studies (Parkunan, 2008) linking ASM and GB99+GB122 to inhibited TCN reproduction. In this study, reduced TCN reproduction was observed in flue-cured tobacco cultivars with and without Ph_p gene resistance to TCN. The ISR inducer GB99+GB122 (Kloepper et al., 2004) consistently lowered TCN reproduction on TCN susceptible (Ph_p-) and resistant (Ph_p+) cultivars, but ASM effects were inconsistent. Consistent reductions by ASM were more closely associated with egg numbers than cyst numbers in Ph_p+ cultivars, suggesting that ASM application alone or in combination with the Ph_p gene hindered nematode egg production more frequently than nematode development.

Resistance to TCN in the Ph_p- flue-cured tobacco cultivar NC567 inhibited feeding site establishment without affecting nematode penetration (Wang et al., 2001). In contrast, GB99+GB122 suppressed nematode penetration on both Ph_p- and Ph_p+ cultivars in our spring 2008 test, while ASM did not influence nematode penetration in either category. Application of ASM to pineapple delayed development and reduced fecundity of reniform and root-knot nematodes, but did not influence penetration, while two bacterial isolates obtained through rhizosphere screening suppressed penetration of Meloidogyne javanica (Treub, 1885) Chitwood, 1949 in Mung bean (*Vigna radiata* (L.) R. Wilcz.) roots (Chinnasri and Sipes, 2005; Siddiqui et al., 2001).

Proposed mechanisms involved in nematode suppression by rhizosphere bacteria include (a) production of secondary metabolites, which directly or indirectly act on plant root exudates, egg hatch, and nematode behavior, or (b) induction of a defense hormone signal leading to induced systemic resistance (Sikora and Hoffmann-Hergarten, 1993). Another possible mechanism of TCN

TABLE 2. Effects of induced resistance elicitors on growth of flue-cured tobacco cultivars with and without the Ph_p gene under greenhouse conditions at the Southern Piedmont Agricultural Research and Extension Center, Blackstone, Va in fall 2007 and spring 2008.*

Cultivars	Treatment	Percent Leaf Yellowing		Fresh Leaf Weight (g)		Fresh Root Weight (g)		Dry Root Weight (g)	
		2007	2008	2007	2008	2007	2008	2007	2008
Ph_p+(NC71 and NC102)	GB99+GB122	23.1c	37.7b	50.0a	79.0b	22.9a	34.2a	3.1a	5.1a
	ASM	44.9a	52.5a	49.7a	84.7a	21.8a	24.9b	2.4b	2.8b
	Untreated	30.4b	26.3b	52.1a	74.8c	22.1a	36.9a	2.8ab	5.6a
Ph_p-(K326 and K346)	GB99+GB122	21.3c	22.4b	46.1b	79.9a	19.5b	38.1a	2.4a	5.8a
	ASM	42.8a	47.4a	48.4a	81.4a	22.1a	26.1c	2.5a	3.2b
	Untreated	28.3b	22.9b	47.8ab	77.8a	21.0ab	34.9b	2.5a	5.5a

* Data presented are non-transformed means of 36 observations across 18 replications and 2 cultivars. Means within a column followed by the same letter are not significantly different according to the Waller-Duncan *t* test (k-ratio =100).

TABLE 3.   Effects of induced resistance elicitors (acibenzolar-*S*-methyl and GB99+GB122) on tobacco cyst nematode (TCN) penetration and development on flue-cured tobacco cultivars with (TCN-resistant; NC71) and without (TCN-susceptible; K326) the *Ph$_p$* gene under greenhouse conditions at the Southern Piedmont Agricultural Research and Extension Center, Blackstone, Va in fall 2007 and spring 2008.

Cultivar	Treatment	Vermiform/ g root*		Vermiform/ whole root*		Swollen/ whole root*		Pyriform/ whole root*		Adult/ whole root*		Parasitic/ whole root*		Swollen/ g root*	
		2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
Ph$_p$+(NC71)	GB99+GB122**	1.9a	2.9b	10.8a	18.8b	1.6b	1.6b	0.0a	0.0a	0.0a	0.0a	1.6b	1.6b	0.3b	0.3b
	ASM**	2.8a	7.4a	13.8a	32.6ab	2.2b	3.0a	0.0a	0.0a	0.0a	0.0a	2.2b	3.0a	0.4b	0.7a
	Untreated	2.4a	9.4a	12.8a	55.8a	10.8a	5.6a	0.4a	0.0a	0.0a	0.0a	11.0a	5.6a	2.1a	0.9a
Ph$_p$-(K326)	GB99+GB122**	2.0a	5.0b	13.2a	25.6b	3.2c	1.8c	0.0b	0.0b	0.0a	0.0a	3.2c	1.8c	0.5c	0.4c
	ASM**	3.0a	7.9ab	14.0a	34.0ab	8.0b	6.0b	0.0b	0.0b	0.0a	0.0a	8.0b	6.0b	1.8b	1.4b
	Untreated	2.5a	10.3a	13.8a	55.4a	27.0a	31.0a	1.2a	4.0a	0.4a	0.4a	28.8a	35.4a	5.6a	6.6a

* Data presented are non-transformed means of five replications. Data were log-transformed [Log$_{10}$(x+1)] prior to statistical analysis. Means within a column followed by the same letter(s) are not significantly different according to the Waller-Duncan *t* test (*k*-ratio =100). Vermiform = second stage juveniles penetrated into the root without any obvious feeding; Parasitic = cumulative number of swollen, pyriform, and adult stages.

** ASM = Acibenzolar-*S*-methyl; GB99+GB122 = *Bacillus subtilis* A13 + *B. amyloliquefaciens* IN937a.

suppression by GB99+GBB122 could be that PGPR may have interfered with production of β-1,4 endoglucanases (cellulases), which have been shown to be upregulated in TCN-infected tobacco (Goellner et al., 2001). The β-1,4 endoglucanases are the cell wall degrading enzymes specifically expressed in root tips, lateral root primordial, and nematode feeding sites (Goellner et al., 2001). If production of this enzyme is hindered in root tips, where the nematode tends to enter the root, around the zone of elongation, nematode intracellular movement into the vascular cylinder of the root may have been blocked. Unsuccessful initial feeding cell development could also lead to suppression of feeding site establishment and thereby arrest the nematode life cycle.

The foliar chlorosis and reduced fresh and dry root weight associated with ASM application suggests that SAR significantly changes allocation of defense-related resources in tobacco. Heil et al., (2000) showed that SAR induced by ASM reduced fitness in wheat by 5-75%. A significant cost was associated with SAR due to the production of several proteins after resistance induction (Ward et al., 1991). Fitness cost associated with induction of resistance correlates well with SAR, but the role of ISR in this process is not clear (Heidel et al., 2004).

Utilizing GB99+GB122 with the *Ph$_p$* gene resistance in *Ph$_p$*+ cultivars reduced TCN reproduction by 99%, compared to a reduction of 94% by resistance through *Ph$_p$* gene alone and 63% for GB99+GB122 (ISR) alone. Combined resistance-PGPR treatments should be compared with nematicide treatments in the field and under commercial agricultural conditions to more thoroughly evaluate the potential of combining *Ph$_p$* gene and induced resistance mechanisms as a viable nematode control alternative to routine nematicide application.

In conclusion, both GB99+GB122 and ASM reduced TCN reproduction, even in the presence of the *Ph$_p$* gene in flue-cured tobacco. Nematode parasitic development was reduced more consistently by GB99+GB122 than by ASM, most likely by inducing ISR. High-throughput, large-scale gene expression studies to compare nematode behavior and feeding site establishment across GB99+GB122 vs. ASM induced *Ph$_p$*+ and *Ph$_p$*- flue-cured tobacco cultivars would help to identify the specific components involved in TCN parasitism and plant resistance response.

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