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ORIGINAL ARTICLE

Nematode ascaroside enhances resistance in a broad spectrum of plant–pathogen systems

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Abstract

Recognition of specific molecule signatures of microbes, including pathogens, induces innate immune responses in plants, as well as in animals. Analogously, a nematode pheromone, the ascaroside ascr#18, induces hallmark plant defences including activation of (a) mitogen-activated protein kinases, (b) salicylic acid- and jasmonic acid-mediated defence signalling pathways and (c) defence gene expression and provides protection to a broad spectrum of pathogens. Ascr#18 is a member of an evolutionarily conserved family of nematode signalling molecules and is the major ascaroside secreted by plant–parasitic nematodes. Here, we report the effects of ascr#18 on resistance in four of the major economically important crops: maize, rice, wheat and soybean to some of their associated pathogens. Treatment with low nanomolar to low micromolar concentrations of ascr#18 provided from partial to strong protection in seven of eight plant–pathogen systems tested with viruses, bacteria, fungi, oomycetes and nematodes. This research may have potential to improve agricultural sustainability by reducing use of potentially harmful agrochemicals and enhance food security worldwide.

KEYWORDS
crop protection, disease resistance, nematode ascarosides, nematode resistance

1 | INTRODUCTION

Increasing population, projected at over nine billion people by 2050, together with rising living standards for hundreds of millions of people and their associated change to more protein-/meat-rich diets, will place increasing demands on food and fibre production. Given that cultivatable land and water are limited worldwide, maximizing production, in part through minimizing crop losses due to disease, is a critical part of the equation for food security (Godfray et al., 2010). Currently, disease management primarily relies on crop rotation, plant breeding for resistance and the use of anti-microbial chemicals and pesticides. All have shortcomings (Jaggard, Qi, & Ober, 2010). Crop rotation often is not economical and/or lacks sufficient effectiveness. Protection based on resistance genes or anti-microbial

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chemicals and pesticides is often lost over time due to selection for resistance in the target population. Thus, despite the use of billions of dollars' worth of synthetic chemicals, tens of billions of dollars of crop loss still occur annually (Oerke & Dehne, 2004). In addition, extensive use of these potentially harmful crop protectants poses environmental and human health risks.

An alternative approach is to enlist the plant's own immune system. Plants, as well as animals (including insects) and fungi, possess innate immunity. Innate immune responses in plants include a cellular influx of Ca^{2+}, generation of reactive oxygen species, activation of a subset of mitogen-activated protein kinases, induction of salicylic acid- and jasmonic acid-mediated defence signalling pathways and extensive transcriptional reprogramming (Pieterse et al., 2012; Robert-Seilaniantz et al., 2011). Innate immune responses are activated by detection of foreign/non-self molecules through plasma membrane-localized pattern recognition receptors. For example, molecules made only by microbes such as bacterial flagellin, lipopolysaccharides and peptidoglycans or fungal chitins have specific molecular patterns (Bittel & Robatzek, 2007; Zipfel, 2014). They are called microbe-associated molecular patterns (MAMPs). Their recognition by pattern recognition receptors activates innate immune responses in host organisms.

Nematodes are ubiquitous in soil and some of the most numerous animals on earth. They parasitize most animals and plants and cause over $150 billion in crop loss per year worldwide (Blaxter & Koutsovoulos, 2015; Singh et al., 2015). Nematode infection of plants induces some of the same innate immune responses as microbes and their MAMPs (Kyndt et al., 2012; Vercauteren, Van Der Schueren, Van Montagu, & Gheysen, 2001). Nematodes produce a unique, evolutionarily conserved family of molecules, called ascarosides, which are used to regulate their development and for communication among themselves and with other organisms (Ludewig et al., 2013; Pungaliya et al., 2009). Ascarosides are derivatives of the dideoxy sugar ascarylose, which is combined with different fatty acid-derived lipophilic side chains and other primary metabolism-derived moieties.

In a previous study, we discovered that the most abundant ascaroside secreted by three different plant–parasitic nematode species, ascr#18, is recognized by plants leading to activation of innate immunity and enhanced disease resistance against a variety of pathogens in several plant species (Manosalva et al., 2015). Here we extend the study to four major crops and their associated pathogens. Together, these two studies demonstrate partial to nearly complete protection in eight plant species against 12 different pathogens and three nematodes. These findings hold great promise for an environmentally friendly and economically sustainable form of disease management.

2 | MATERIALS AND METHODS

2.1 | Soybean pathosystems

Soybean (*Glycine max* (L.) Merr.) cultivars Essex, Williams and Harosoy were grown in the greenhouse with day and night temperatures of 25°C and 20°C, respectively. Soybean plants at V1 stage were sprayed with 5 ml/plant ascr#18 (0 μM, 0.01 μM 0.1 μM, 1 μM and 10 μM supplemented with 0.01% (w/v) Silwet L-77 as a surfactant) at 48 hr and again 24 hr prior to pathogen inoculation. All soybean pathosystems experiments were done at least twice with similar results.

For the Soybean Mosaic Virus strain G5 (SMV-G5) assay, virus-infected plant tissue was homogenized in 0.01 M phosphate buffer, mixed with a small amount of carborundum and rub-inoculated on leaves of plants (cv. Essex) at the V1 stage. Leaf tissue from the inoculated and uninoculated, systemic leaves were collected at different time intervals (0, 7, 10 dpi for inoculated leaves; 7 and 10 dpi for uninoculated leaves) and analysed by immunoblotting using SMV coat protein-specific antibodies.

For the *Pseudomonas syringae* pv *glycinea (Psg)* assay, bacteria were grown on King's B medium at 29°C supplemented with 50 mg/ml rifampicin plus 50 mg/ml kanamycin. First, trifoliate leaves of V1 stage plants (cv. Williams) were infiltrated with bacterial suspensions of 1×10^5 CFU/ml in 10 mM $MgCl_2$ plus 0.01% Silwet L-77. Mock inoculations were carried out with 10 mM $MgCl_2$ in 0.01% Silwet L-77. Bacterial growth was monitored by dilution plating of homogenized leaf disks excised using a 1 cm cork borer at 0 and 4 days post inoculation. At least four inoculated plants per treatment were sampled individually.

For the *Phytophthora sojae* (race 1 or race 3) assays, cultivar Harosoy was used as the host instead of Essex or Williams. *P. sojae* was grown on V8 agar at 25°C in the dark. Soybean inoculations with *P. sojae* were done at V1 stage as previously described (Kachroo et al., 2008). Briefly, soybean seedlings were inoculated by placing a small amount of mycelium in a vertical slit wound (approximately 1 cm in length) made 2 to 3 mm below the first trifoliate leaf, and then covering the inoculated wound with parafilm. Mock inoculations were carried out with agar plugs without mycelia. Disease progression was measured as the number of plants killed in response to *P. sojae* infection.

2.2 | Rice pathosystem

Rice (*Oryza sativa* cv. Kitaake) seeds germinated in petri dishes for 7 days were transplanted to pots containing 50:50 mixture of Promix potting mix and soil amendment Profile Greens Grade and grown in a greenhouse at 85% relative humidity with 16-hr light /8-hr dark and 30°C day/25°C night. Two weeks after transplanting, plants received fertilizer (Peter's 15-5-15) two times per week. Four-week-old plants were sprayed with approximately 50 ml of one of the five treatments (0.01 μM ascr#18, 0.1 μM ascr#18, 1 μM ascr#18, 10 μM ascr#18 and water (mock)) at 48, 36, 24 and 12 hr prior to pathogen inoculation. Treatment solutions contained a surfactant (0.05% Silwet) and were normalize for the small amounts of ethanol present in the ascr#18 solution. Plants were inoculated with *Xanthomonas oryzae* pv. *oryzae* strain PXO86. Cultures of *X. oryzae* pv. *oryzae* PXO86 were incubated at 28°C on peptone-sucrose agar medium for 24 hr, then bacteria scraped from the plate were suspended in sterile water at an optical density (OD600) of 0.2 (1×10^8 CFU/mL). Two leaves from each plant were inoculated by dipping a scissors into the bacterial suspension before cutting each leaf four cm from

the tip (Kauffman, Reddy, Hsieh, & Merca, 1973). Lesion lengths were measured 14 days post inoculation. A two-way ANOVA was performed to evaluate the effects of ascr#18. Data were analysed using JMP Pro version 12.0.1. Differences between treatments were determined using Tukey's HSD.

2.3 | Wheat pathosystem

Wheat *(Triticum aestivum* cv. Kanzler*)* plants were grown under 16-hr/8-hr light/dark conditions at 22°C (Vötsch plant chamber, 12,000 Lux). Seven-day-old plants were sprayed 24 hr prior to inoculation with 0.01 μM ascr#18, 0.1 μM ascr#18, 1 μM ascr#18 and 10 μM ascr#18 in an aqueous solution containing 0.1% ethanol. Plants on 1.0 m^2 trays were sprayed with 50 ml of the respective ascr#18 solutions. Control plants were mock treated with 0.1% ethanol. Plants were spray inoculated with 2.5×10^6 conidia/ml of *Zymoseptoria tritici* in water containing 0.1% Tween20 until run-off. Plants were incubated and placed in closed plastic boxes for four days at 100% relative humidity before transfer to open boxes. Disease symptoms were scored 21 days post inoculation according to the scheme described (Moll et al. 2010). *Zymoseptoria tritici* (GWH 5314) was cultured on ISP2 (International Streptomyces Project) medium (yeast extract 0.4%, malt extract 1%, dextrose 0.4%, agar–agar 2%, pH 7.2). Plates were incubated at 22°C under 8-hr/16-hr light/dark (Philips HF, 6,200 Lux). Conidia were harvested from 10-day-old cultures with a sterile glass rod and sterile 0.1% Tween 20-containing water, before filtering through one layer of sterile mira cloth. The relative amount of fungal DNA was measured using qRT-PCR. DNA was extracted using the CTAB method (Chen & Ronald, 1999). Primers used for assessing expression of the fungal *Cytochrome b* gene: (fwd-CCCTAGAACATTAACATGAACAATCG; rev-CAATAAGTTAGTTATAACTGTTGCCC). Primers for expression analysis of the barley *Ubiquitin* gene: (fwd- ACCCTCGCCGACTACAACAT; rev-CAGTAGTGGCGGTCGAAGTG). Transcript levels were determined via the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001) by normalizing the amount of target transcript to the amount of plant *Ubiquitin* transcript.

2.4 | Maize pathosystems

Maize (*Zea mays* cv. W64A-N) plants were grown in a growth chamber with a light cycle of 16-hr light/8-hr dark at 24℃. *Cochliobolus heterostrophus* strain C4 was grown on complete medium with xylose under a 16-hr light/8-hr dark regimen at 23℃ as previously described (Leach, Lang, & Yoder, 1982; Wu et al., 2012). *Setosphaeria turcica* strain 28A was grown on lactose casein agar (Xue et al., 2013) under a 12 hr light/12 hr dark regimen at 23℃. Two to three-week

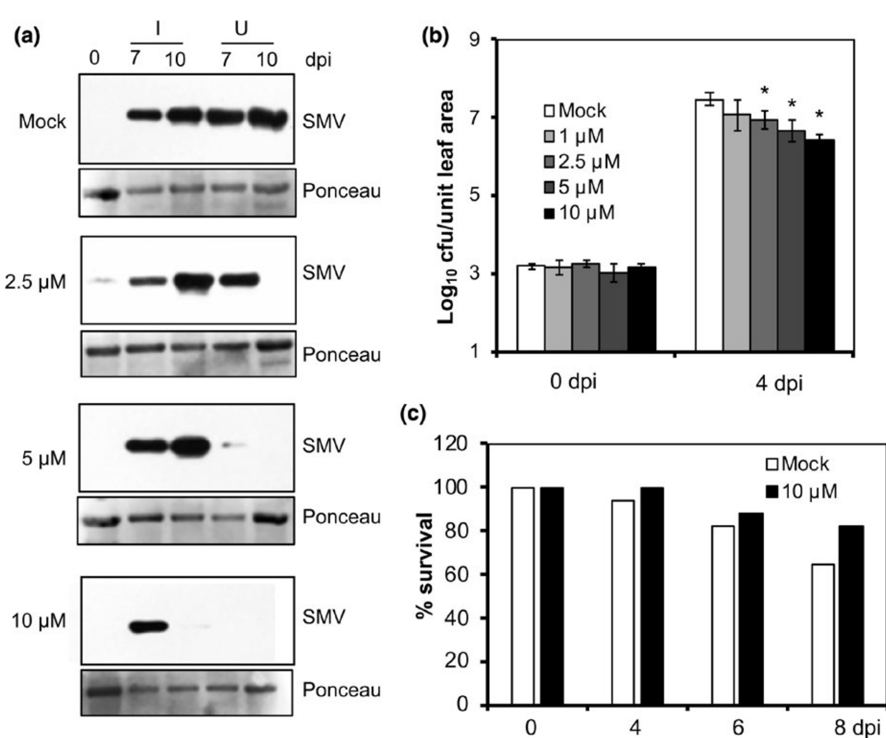
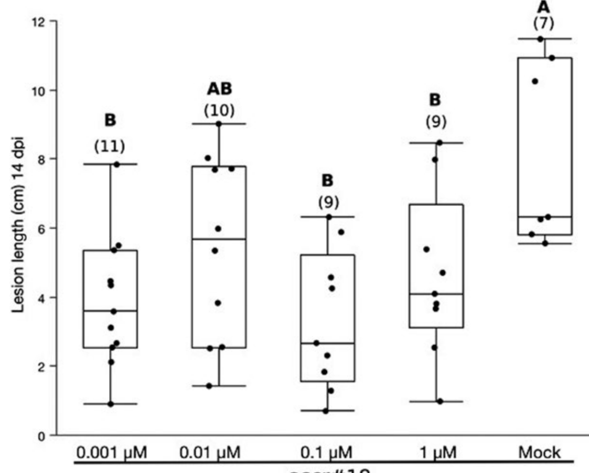


FIGURE 1 Ascr#18 treatment of soybean plants enhanced resistance to viral, bacterial and oomycete pathogens. (a), (b) and (c) Soybean plants (cv. Essex) at the V1 stage were sprayed with the indicated concentrations of ascr#18 followed by inoculation with (a) Soybean Mosaic Virus (SMV), (b) *Pseudomonas syringae* pv. *glycinea* (*Psg*) and (c) *Phytophthora sojae* (race R1). (a) SMV accumulation was monitored in the inoculated (I) and uninoculated systemic (U) leaves at 7 and 10 days post inoculation using immunoblot analysis with SMV coat protein-specific antibodies. Ponceau staining was used as control for protein levels. (b) *Psg* growth was monitored at 0 and 4 dpi and is presented as LOG_{10} value of colony forming units (CFU) per unit leaf area. Asterisks denote significant difference from control plants treated with only 0.01% Silwet L- 77 ($p < 0.001$). (c) Percentage of plant survival was monitored 2, 4, 6 and 8 dpi with *P. sojae*. All experiments were done at least twice with similar results



ascr#18 concentration	Mean lesion length (cm)
0.001 μM	3.85691
0.01 μM	5.41020
0.1 μM	3.31378
1 μM	4.62567
Mock (0 μM)	8.08486

FIGURE 2 Ascr#18 treatment weakly suppressed bacterial blight on rice caused by *Xanthomonas oryzae* pv.*oryzae* strain PXO86. Four-week-old rice plants (cv. Kitaake) were sprayed with the indicated concentrations of ascr#18 at 48, 36, 24 and 12 hr prior to inoculation. Length of lesions at 14 days post inoculation was used to quantify disease severity. Different letters indicate statistically significant difference between them as determined by Tukey's HSD (*$p < 0.05$). Numbers in parentheses are *n*. Results from a second experiment also showed overall reduction in lesion length, but due to large standard deviations the differences from the mock control were not statistically significant

old plants were sprayed with 2 ml/plant of ascr#18 (0.01 µM, 0.1 µM, 1 µM and 10 µM) in 0.02% Tween 20 and 0.001% ethanol, 48 hr prior to inoculation. Water containing 0.02% Tween 20 and 0.001% ethanol served as the mock control. Plants were then inoculated by spraying each plant with 2 ml of spore suspension at a concentration of 5,000 spores/mL in 0.02% Tween 20. Disease symptoms on the 3rd true leaf were assessed 4 days post inoculation in the case of *C. heterostrophus* and at 9–15 days in the case of *S. turcica*. Six plants were used per treatment. *C. heterostrophus*-induced lesion numbers were measured using ImageJ software (NIH, https://imagej.nih.gov/ij/) and differences evaluated using one way ANOVA (https://www.socscistatistics.com/tests/Default.aspx). The experiments were done twice with *C. heterostrophus* and repeated three times with *S. turcica*.

2.5 | Tomato pathosystem

Tomato (*Solanum lycopersicum* cv. Moneymaker) seeds were sown in a mixture of sand and Turface (3:1) in Cone-tainers (3.8 cm x 21 cm; Stuewe & Sons, Inc., Tangent, OR) and maintained under greenhouse conditions as previously described (Liu & Williamson, 2006). At 4 weeks after planting, Cone-tainers were immersed in indicated concentrations of ascr#18 or control (water with the same % ethanol) for 48 hr prior to inoculation. Each plant was inoculated with 500 infective *Meloidogyne hapla* strain VW9 J2 juveniles. Five weeks after inoculation, the number of eggs per plant was quantified as previously described (Liu & Williamson, 2006). The experiment was laid out in complete randomized design with each treatment replicated five times.

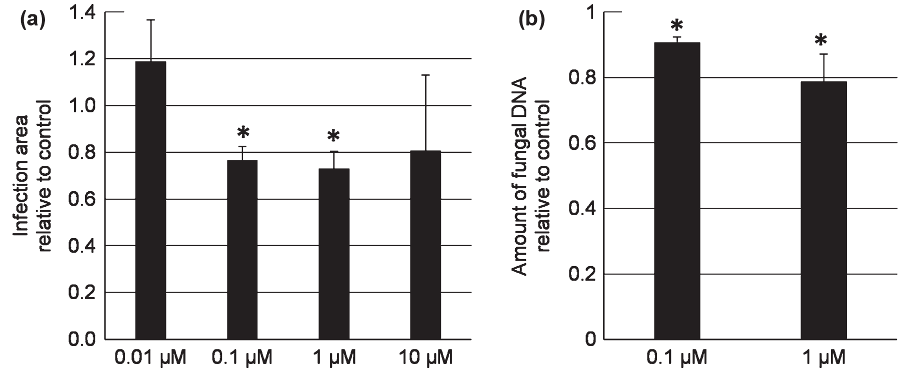


FIGURE 3 Suppression of disease symptoms and multiplication of *Zymoseptoria tritici* on wheat. Seven-day-old wheat plants (cv. Kanzler) were sprayed with aqueous 0.1% ethanol containing from 0 µM up to 10 µM ascr#18 and 24 hr later inoculated with *Z. tritici*. At 21 dpi infect area (a) and fungal multiplication (b) were determined relative to the control treated with only aqueous 0.1% ethanol. The amounts of fungal DNA, as determined by qRT-PCR, were used as a measure of *Z. tritici* multiplication. Best protection was obtained by treatment with 0.1 µM and 1.0 µM ascr#18, for which the fungal multiplication results are presented. Error bars represent SE for data from three independent experiments. Asterisks indicate statistically significant differences to 0.1% ethanol control treatment. (*$p < 0.05$ in *t* test)

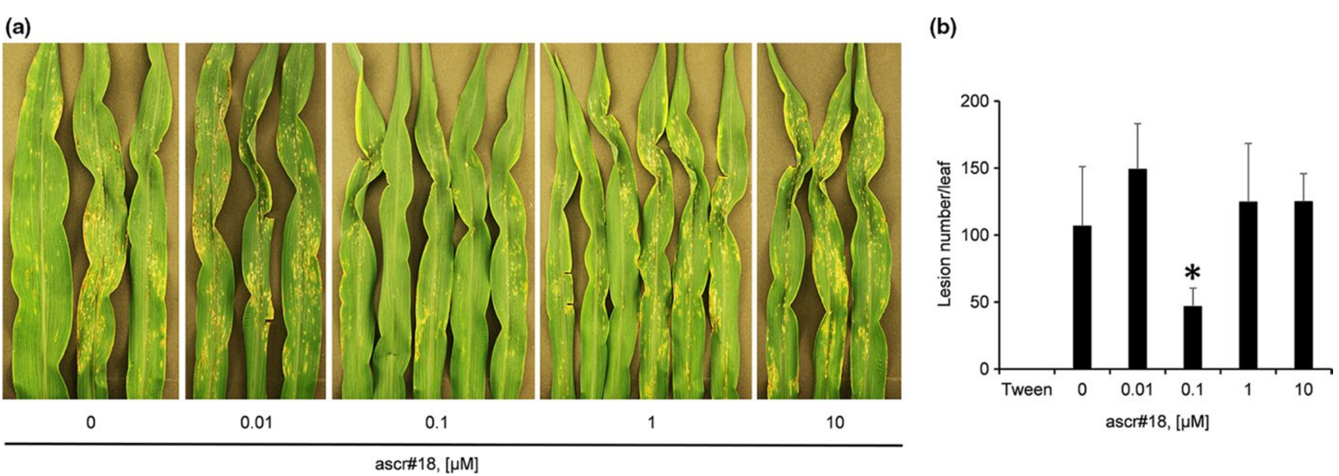


FIGURE 4 Ascr#18 treatment of maize suppressed southern corn leaf blight caused by *Cochliobolus heterostrophus*. (a) Two- to three-week-old maize plants (cv. W64A-N) were sprayed with the indicated concentrations of ascr#18 48 hr prior to inoculation with *C. heterostrophus* strain C4. Disease symptoms were assessed 4 dpi. (b) Lesion numbers were measured using ImageJ software and differences evaluated using one way ANOVA. The experiments were done twice with similar results. (*$p < 0.05$)

3 | RESULTS

Soybean is the major high-protein, oil-rich seed crop worldwide. It is used primarily as a food source for humans and an animal feed supplement. Annual worldwide production in 2017/2018 is estimated to be 340 million metric tons with the US producing approximately 120 million metric tons (US Department of Agriculture). Soybean is attacked by a wide variety of pathogens that cause significant yield losses. Given that the ascr#18 provides protection to diverse plant species (Manosalva et al., 2015), we suspected that ascr#18 may also provide protection to soybean against its pathogens. We tested whether foliar treatment with ascr#18 would protect soybean against three different classes of pathogens—viruses, bacteria and oomycetes. V1 stage plants were sprayed twice with several different concentrations of ascr#18 from 0.01 µM to 10 µM prior to inoculation with the pathogens. Ascr#18 provided strong protection against Soybean Mosaic Virus (SMV). Treatment with increasing concentrations from 2.5 µM to 10 µM partially suppressed virus proliferation in the inoculated leaves, as measured by immunoblot analyses with SMV coat protein-specific antibodies (Figure 1a). The spread of the pathogen to uninoculated leaves on the plant and/or replication in those leaves was severely repressed, with little or no detectable virus accumulation in plants treated with 5 µM or 10 µM ascr#18. Concentration-dependent ascr#18 inhibition of multiplication of an avirulent strain of the hemi-biotrophic bacterial pathogen *P. syringae* pv *glycinea* was also observed (Figure 1b). Ten µM ascr#18 reduced *P. syringae* pv *glycinea* growth by ten-fold as compared to control.

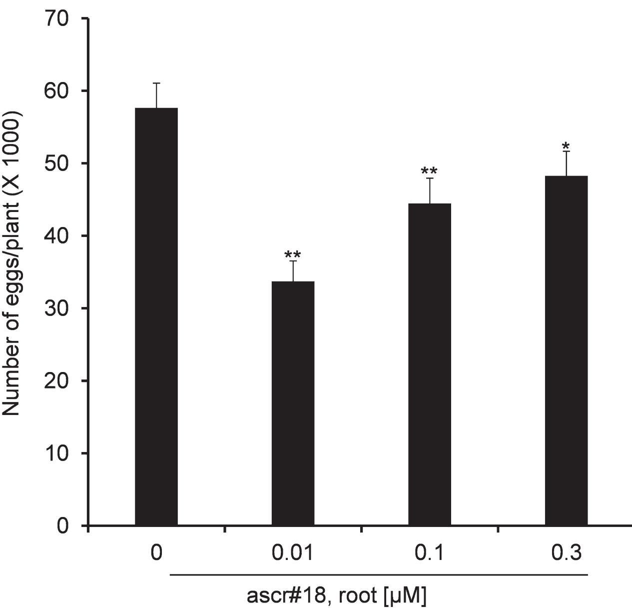


FIGURE 5 Suppression of nematodes reproduction on tomato. Four-week-old tomato plants (cv. Moneymaker) were sprayed with the indicated concentration of ascr#18 48 hr prior to inoculation with 500 freshly hatched J2 of *Meloidogyne hapla* strain VW9. Five weeks post inoculation the number of nematode eggs per plant were determined. Asterisks denote significant difference from the water-treated control. (*$p < 0.05$, **$p < 0.01$)

Species of *Phytophthora* are among the most destructive plant pathogens. For example, *P. infestans* caused the Great Irish Potato Famine of the 1840s and remains a major threat to the cultivation of potato and tomato. We previously showed that ascr#18 partially suppressed late blight caused by *P. infestans* on both potato and tomato (Manosalva et al., 2015). Its relative *P. sojae* is the most severe pathogen of soybean, killing infected plants within days. Since treatment with 10 µM ascr#18 provided the best protection against SMV and *P. syringae* pv *glycinea*, we tested its effect on the survival of *P. sojae*-inoculated soybean (Figure 1c). By eight days post inoculation, only 65% of mock-treated plants survived. That contrasted with a 50% increase in survival rate of 83% for ascr#18-treated plants.

Rice is the third largest crop by tonnage after maize and sugarcane and the foremost staple food worldwide, supporting calories

TABLE 1 Effect of ascr#18 treatments in different pathosystems

Host	Pathogen/Pest	Pathogen type	Level of protection	Optimal concentration
Soybean	Soybean Mosaic Virus	Biotrophic virus	S	10 μM
	Pseudomonas syringae pv. glycinea	Hemi-biotrophic bacterium	MS	10 μM
	Phytophthora sojae[a]	Hemi-biotrophic oomycete	S	10 μM
Rice	*Xanthomonas oryzae pv. oryzae*	Biotrophic bacterium	W	0.1 μM
Maize	*Cochliobolus heterostrophus*	Necrotrophic fungus	M	0.1 μM
	Setosphaeria turcica	Hemi-biotrophic fungus	None	N/A
Wheat	*Zymoseptoria tritici*	Hemi-biotrophic fungus	M	0.1 μM
Barley	*Blumeria graminis f sp hordei*[b]	Biotrophic fungus	MS	0.3 μM
Potato	*Phytophthora infestans*[b]	Hemi-biotrophic oomycete	M	0.01 μM
Tomato	*Phytophthora infestans*[b]	Hemi-biotrophic oomycete	M	0.01 μM
	Pseudomonas syringae pv. tomato[b]	Hemi-biotrophic bacterium	M	0.01 μM
	Meloidogyne hapla	Biotrophic root-knot nematode	M	0.01 μM
Arabidopsis	Turnip Crinkle Virus[b]	Biotrophic virus	S	1.0 μM
	Pseudomonas syringae pv. tomato[b]	Hemi-biotrophic bacterium	M	1.0 μM
	Heterodera schachtii[b]	Biotrophic cyst nematode	M	0.01 μM
	Meloidogyne incognita[b]	Biotrophic root-knot nematode	M	0.01 μM

Note. S: strong protection; MS: moderately strong protection; M: modest protection; W: weak protection
[a]Only concentration that was tested. [b]Data reported in Manosalva et al. (2015).

for almost half of the world's population (UN Food and Agriculture Organization 2017). Bacterial blight caused by *X. oryzae* pv. *oryzae* is the most important bacterial pathogen of rice, causing losses ranging from 10%–50% in Asian and Southeast Asian countries (Ou, 1985). Ascr#18 was tested over a range of 0.01 μM to 10 μM. Treatment with 0.1 μM ascr#18 reduced disease severity most effectively (Figure 2).

After rice, wheat is the most important food crop. Diseases are responsible for substantial production losses in wheat. *Septoria tritici* blotch, caused by the hemi-biotrophic fungal pathogen *Zymoseptoria tritici*, is the most severe foliar disease of wheat (Jørgensen et al., 2014). Ascr#18 was tested over a range of 0.01 μM to 10 μM. 0.1 μM and 1.0 μM ascr#18 reduced disease symptoms about 25% (Figure 3a) and fungal growth 10% to 20% (Figure 3b); treatment with the other concentrations of ascr#18 provided little or no protection.

Maize is the largest crop worldwide and is the foremost high-energy feed stock. The estimated 2017/2018 US production is almost 371 million metric tons, more than a third of the estimated worldwide production of 1,003 million metric tons (US Department of Agriculture). *C. heterostrophus* is a necrotrophic fungal pathogen, highly specific for maize, on which it causes southern corn leaf blight. Ascr#18 was tested over a range of 0.01 μM to 10 μM. Treatment with 0.1 μM provided modest protection (Figure 4).

Crop losses to plant–parasitic nematodes are estimated at more than $150 billion annually (Singh et al., 2015). We previously reported that ascr#18 provided modest protection of *Arabidopsis* against the cyst nematode *Heterodera schachtii* and root-knot nematode *Meloidogyne incognita* (Manosalva et al., 2015). We extended that study to root-knot nematode *M. hapla* on tomato and found that sub-micromolar amounts of ascr#18 also provided protection in this plant–pathogen/pest system (Figure 5).

4 | DISCUSSION

In this study, we extended our assessment of ascr#18-mediated protection to four additional crops against eight pathogens/pests, including one virus, two bacteria, three fungi, one oomycete and one nematode. Treatment with ascr#18 provided partial to strong protection in seven of the eight plant–pathogen systems. The exception was maize challenged with *Setosphaeria turcica*, in which ascr#18 treatment failed to provide any protection.

These results are consistent with those in our previous report (Manosalva et al., 2015), in which we demonstrated protection in four other plant species (barley, potato, tomato and *Arabidopsis*) against another virus (Turnip Crinkle Virus), bacterium (*Pseudomonas syringae* pv. *tomato*), fungus (*Blumeria graminis* f sp. *hordei*), oomycete (*P. infestans*) and two nematodes (*H. schachtii* and *M. incognita*). Taken together, ascr#18 enhanced resistance in 15 of these 16 plant–pathogen/pest systems including eight plants species and 15 pathogens/pests (Table 1). Biotrophic, hemi-biotrophic and necrotrophic pathogens were represented in both studies. The concentration optimum of ascr#18 for maximum protection varied form 0.01 μM to 10 μM and was primarily dependent on the plant species, and much less so on the pathogen. While 0.1 μM and/or 1 μM ascr#18 generally provided the best protection in most species, maximum protection in the closely related tomato and potato was afforded with 0.01 μM. In contrast, 10 μM ascr#18 provided maximum protection in soybean.

In this study, ascr#18 was applied via spraying for protection against microbial pathogens and via root dipping for protection against nematodes. We previously showed that ascr#18 administration to roots, as well as leaves, induced innate immune responses in both roots and leaves and provided protection (Manosalva et al.,

2015). In a forthcoming report, we demonstrate that treatment of seeds with ascr#18 enhances resistance in several important crop plants against a variety of pathogens, including soil-borne pathogens. In addition, it will add to the list four more pathogens against which ascr#18 provides protection.

Ascarosides, which consist of a fatty acid-derived side chain attached at the α position to the dideoxysugar ascarylose, bear some structural similarity to bacterial rhamnolipids, which consist of lipophilic fatty acid side chain(s) attached at the α position to one or two rhamnose sugar moieties. Rhamnolipids function similar to MAMPs, inducing cellular influx of Ca^{2+}, generation of reactive oxygen species, activation of mitogen-activated protein kinases, induction of salicylic acid- and jasmonic acid- mediated defence signalling pathways, and activation of defence gene expression (Sanchez et al., 2012; Varnier et al., 2009; Vatsa et al., 2010). Due to their amphiphilic structure, rhamnolipids have detergent-like properties, and it has been suggested that rhamnolipids might activated immune responses by membrane disruption (Sanchez et al., 2012). In contrast, ascarosides are not amphiphilic and thus have no detergent-like properties, and the strong activation of innate immune responses at low concentrations suggests that their perception is receptor mediated.

In summary, ascr#18 treatment by a variety of routes induces innate immunity, which provides weak to strong protection in all major crops tested against a broad range of pathogens as well as both cyst and root-knot nematodes. Use of this natural compound at low nanomolar to low micromolar concentrations holds great promise for crop protection in an economical, environmentally friendly manner for improved agriculture sustainability.

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CONFLICT OF INTEREST

Authors Murli Manohar, Frank Schroeder and Daniel Klessig are co-founders of Ascribe Bioscience, which was partially founded on ascr#18-induced plant immunity reported in Manosalva et al. (2015).

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