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ORIGINAL PAPER

Cropping history affects nodulation and symbiotic efficiency of distinct hairy vetch (*Vicia villosa* Roth.) genotypes with resident soil rhizobia

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Abstract Compatible rhizobia strains are essential for nodulation and biological nitrogen fixation (BNF) of hairy vetch (*Vicia villosa* Roth, HV). We evaluated how past HV cultivation affected nodulation and BNF across host genotypes. Five groups of similar HV genotypes were inoculated with soil dilutions from six paired fields, three with 10-year HV cultivation history (HV+) and three with no history (HV−), and used to determine efficiency of rhizobia nodulation and BNF. Nodulation was equated to nodule number and mass, BNF to plant N and *Rhizobium leguminosarum* biovar *viceae* (Rlv) soil cell counts using qPCR to generate an amplicon of targeted Rlv *nodD* genes. Both HV cultivation history and genotype affected BNF parameters. Plants inoculated with HV+ soil dilutions averaged 60 and 70 % greater nodule number and mass, respectively. Such plants also had greater biomass and tissue N than those inoculated with HV− soil. Plant biomass and tissue N were strongly correlated to nodule mass (r^2=0.80 and 0.50, respectively), while correlations to nodule number were low (r^2=0.50 and 0.31, respectively). Although hairy vetch rhizobia occur naturally in soils, past cultivation of HV was shown in this study to enhance nodulation gene-carrying Rlv population size and/or efficiency of rhizobia capable of nodulation and N fixation.

Keywords Nodulation · Symbiosis · Biological nitrogen fixation (BNF) · Rhizobia · *Rhizobium leguminosarum* biovar *viciae* (Rlv) · Hairy vetch

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Introduction

Hairy vetch (*Vicia villosa* Roth; HV) is widely used in agroecosystems as a legume cover crop and green manure, with benefits including erosion control, weed and pest suppression, and soil N fertility improvement (Utomo et al. 1990; Power et al. 1991; Teasdale and AbdulBaki 1997; Kuo and Sainju 1998; Lu et al. 2000; Anugroho et al. 2009; Campiglia et al. 2010). Native to Europe or Western Asia (Undersander et al. 1990), HV has been domesticated in various geographic areas, including the USA where it is commonly used in organic agriculture as a N source. Varieties with specific plant characteristics have been developed for different geographical regions, such as the Madison variety developed in Nebraska with increased cold tolerance, and Purple Bounty developed for early maturity and high biomass production (Maul et al. 2011).

The degree of legume nodulation is known to be a powerful determinant of BNF in legume species (Ballard et al. 2004; Chemining'wa and Vessey 2006; Drew and Ballard 2010). Inoculation with efficient rhizobia at planting is often recommended in environments where compatible rhizobia are absent, soil rhizobial population density has been reduced, or where rhizobia are shown to be less effective (Chemining'wa and Vessey 2006). In some cases, however, resident soil rhizobia, including native rhizobia and those naturalized through past inoculation, have been shown to

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compete for nodule occupancy with introduced rhizobial strains, impacting inoculation success (Thies et al. 1991; Toro 1996; Denton et al. 2002). Resident rhizobia vary in their capacity to nodulate and fix N with host plants. For example, symbiotic efficiency of *Medicago sativa* and *Trifolium* species with resident rhizobia has been measured to range from −6 to 82 % and from 10 to 130 %, respectively, of that resulting from recommended commercial inoculants (Ballard and Charman 2000; Drew and Ballard 2010).

Total biomass N contributed by HV ranges from 100 to 240 $kg ha^{-1}$ (Parr et al. 2011; Wagger 1989) depending in part on the efficiency of the rhizobia symbiosis (Toro 1996). An understanding of how HV interacts with resident soil rhizobia is critical to improve N contribution to agricultural systems. Hairy vetch is nodulated specifically by *Rhizobium leguminosarum* biovar *viceae* (Rlv), the same rhizobia species nodulating pea (*Pisum sativum*), faba bean (*Vicia faba*), and common vetch (*Vicia sativa*). While some studies show Rlv inoculation to improve HV productivity (Chemining'wa and Vessey 2006; Toro 1996), others have reported lack of Rlv inoculation response (Ballard et al. 2004). From these contrasting observations emerge questions about the effect of cropping practices, particularly past planting history and use of host legume, on the infective ability and symbiotic efficiency of resident Rlv populations.

Many soil properties including pH, texture, and temperature, as well as management practices such as inoculation, contribute to variation in BNF across the landscape (Drew and Ballard 2010; Toro 1996), with legume genotype and interaction with resident rhizobia being a critical consideration. Previous studies have shown large variation in BNF efficiency both between (Kitou et al. 2010; Parr et al. 2011) and within (Drew and Ballard 2010; Unkovich et al. 1997) cover crop species. Efficiency of BNF has been shown to be higher in legume genotypes that are compatible with a wide range of rhizobial strains (Drew and Ballard 2010).

Compatible resident rhizobia can exist in fields that have never been cultivated to a given host legume due to the presence of native legume species, transfer from adjacent fields, or as part of the native microbiological soil community (Meade et al. 1985; Thies et al. 1991). In the absence of a host plant, rhizobia have saprophytic competence allowing them to survive in soil for an extended period of time. Introducing a legume host to an environment lacking past or recent cultivation history presents an alternative survival strategy for already existing rhizobia populations. We understand little about the compatibility of resident HV rhizobia with introduced hosts, or how past HV cultivation affects nodulation and N fixation efficiency. This information is particularly lacking for hairy vetch cover crop cultivars, often planted for mutiple consecutive years in organically managed systems that commonly use this cover crop for N delivery. The objectives of our study were to: (1) determine the effect of hairy vetch cultivation history on nodulation and BNF efficiency of resident Rlv, (2) determine the nodulation and BNF efficiency of distinct hairy vetch genotypes with resident Rlv, and (3) quantify the effects of past cultivation on HV compatible rhizobia population size. Our guiding hypotheses were that fields with a history of HV cultivation would have higher nodulation, BNF efficiency, and larger population sizes than fields without cultivation history.

Materials and methods

Site selection and soil sampling

Soil samples were taken from three farms with a history of hairy vetch cultivation. From each farm, one field with history of hairy vetch planting (designated HV+) and one never planted to hairy vetch nor observed to have wild hairy vetch or other legumes (grower personal communication; designated HV−) were sampled. Farm selection criteria were defined as having at least five seasons of HV since 1990 and similar soil pH between sampled fields of the same farm. Farm sites were located in Graham, Cedar Grove, and Ivanhoe, North Carolina. The Cedar Grove field was inoculated with Rlv each season hairy vetch was planted, Graham was never inoculated, and Ivanhoe had not been inoculated since 2004 (Table 1). Sampling was carried out in March 2010, when all fields with hairy vetch history were currently planted to HV, in combination with pea and rye in Graham, rye in Cedar Grove, and monoculture in Ivanhoe.

Forty soil cores (2.5 cm dia.×15 cm depth) were randomly collected from each field and mixed to form a composite sample. Soil samples were kept cool during transport to the lab and thereafter stored at 4 °C until processing. All sampling tools were sterilized with 75 % ethanol prior to sampling and during sampling and handling, taking precautions to avoid cross-contamination of soils of different fields and sites. Soil sub-samples for inorganic N determination were dried at 45 °C for 2 days, ground, and sieved to pass a 2-mm screen. Soil inorganic N (NH_4^+ and NO_3^-) was determined colorimetrically with a QuikChem 2000 flow injection autoanalyzer (Lachat Instruments, Loveland CO) after soil samples were extracted with 1 M KCl at soil-to-extractant ratio 1:5 and filtered through Whatman #42 filter papers. Soil samples were also analyzed for pH, cation exchange capacity, base cations, base saturation, phosphorus, manganese, zinc, copper, and humic matter (Table 2).

Hairy vetch genotypes

Nodulation assessment of resident soil Rlv was evaluated using five groups of distinct hairy vetch genotypes, each group comprising two closely related genotypes (Maul et al. 2011).

Table 1 Characteristics of fields from which soils were collected for inoculation of the ten distinct HV genotypes, modified from Mothapo et al. 2013

Site	Soil type	Field history	Status during sampling	Last inoculation with Rlv	Other legume cover crops used
Graham	Appling Sandy Loam (Fine, kaolinitic, thermic Typic Kanhapludults)	HV+	Hairy vetch, pea, rye	Never been inoculated	Cowpea, crimson clover, pea
		HV–	Asparagus	–[a]	–[a]
Cedar Grove	Appling Sandy Loam (Fine, kaolinitic, thermic Typic Kanhapludults)	HV+	Hairy vetch, rye	Every season HV was planted	Crimson clover
		HV–	Grass	–[a]	–[a]
Ivanhoe	Chipley Sand (Thermic, coated Aquic Quartzipsamments)	HV+	Hairy vetch	2004	Crimson clover
		HV–	Fallow weeds	–[a]	–[a]

[a] Field does not have history of legume cropping or growth, therefore, no inoculation

Genotypes were previously collected from Afghanistan (two genotypes), Greece, Iran (two genotypes), Turkey (two genotypes), USDA varieties including *Purple Bounty* and *Purple Prosperity* early maturity varieties (USA-MD 1 and USA-MD 2, respectively), and the *Madison* variety from Nebraska, USA-NE (Table 3). Seeds for Afghanistan, Greece, Iran, and Turkey genotypes were obtained from National Plant Germplasm System (Washington State University, Pullman, WA), and seeds for USA genotypes were obtained from USDA-ARS Sustainable Agriculture Systems Lab (Beltsville, MD).

Experimental design and plant germination

The ten HV genotypes were used to trap soil rhizobia from collected soils over a period of 6 weeks in a growth chamber. Two coupled magenta units (PlantMedia, Dublin, OH) were used (Tlusty et al. 2004); the bottom unit contained N-free nutrient solution (Broughton and Dilworth 1971) and the top unit equal volumes of sand and vermiculite thoroughly mixed, drilled at the bottom with inserted cotton wick to source water and nutrients from the bottom unit. Assembled magenta units were sterilized by autoclaving at 121 °C for 15 min.

Hairy vetch seeds were surface sterilized with 3 % sodium hypochlorite, rinsed five times in sterile deionized water, placed on a sterilized germination paper in Petri dishes, and left to germinate at room temperature for 6 days. Genotypes Turkey 1, Turkey 2, Iran 1, and Greece were scarified by soaking seeds in 80 % H_2SO_4 for 30 min, then rinsing five times with deionized water prior to sterilization to improve germination. Each field treatment (HV+and HV−) included ten experimental units made up of the HV genotypes, with two hairy vetch seedlings originally planted per magenta unit. Each seedling was inoculated with 500 μl of a soil dilution prepared by mixing 20 g of the reserved soil with 80 ml of 0.85 % (*w*/*v*) NaCl solution (Bala et al. 2001). For each field, four soil dilution repetitions were prepared as inoculants in order to minimize dilution variability for consistent treatment effect. Due to growth chamber space constraints, the experiment was divided into two runs separated by time period. Run 1 was established in May/June

Table 2 Soil chemical properties for the six fields used in the study to test efficiency of resident rhizobia in nodulation of hairy vetch

Soil property	Graham		Cedar Grove		Ivanhoe	
	HV+	HV−	HV+	HV−	HV+	HV−
pH (H_2O)	6.5	6.1	6.6	5.7	5.7	5.4
P ($mg\,dm^{-3}$)	67.00	103.30	328.20	77.70	497.30	658.00
K (meq 100 cm^{-3})	0.34	0.27	0.22	0.22	0.82	0.85
Ca (meq 100 cm^{-3})	4.30	3.18	10.05	2.84	7.63	4.32
Mg (meq 100 cm^{-3})	1.04	1.12	2.03	1.40	1.92	1.38
Na (meq 100 cm^{-3})	0.0	0.0	0.1	0.0	0.1	0.1
Mn ($mg\,dm^{-3}$)	95.2	69.8	31.4	32.6	12.0	9.6
Zn ($mg\,dm^{-3}$)	15.5	18.9	7.1	2.3	18.2	13.9
Cu ($mg\,dm^{-3}$)	2.7	1.4	2.7	1.0	1.1	10.1
Base saturation (%)	89.0	79.0	94.0	79.0	85.0	64.0
Cation exchange capacity	6.4	5.8	13.1	5.7	13.3	10.1
Humic matter ($g\,100\ cm^{-3}$)	0.41	0.32	0.18	0.41	0.86	3.28
Inorganic N (%)	2.96	9.58	4.42	5.92	2.97	5.54

Table 3 Nodulation and biomass production of the hairy vetch genotypes

Group	Hairy vetch genotype	Nodule number (plant^{-1})	Nodule mass (mg plant^{-1})	Shoot biomass (mg plant^{-1})	Shoot N conc. (%)	Total shoot N (mg plant^{-1})	Sample size (n)
1	Turkey 1	32b	18.8c	390.3a	4.01b	15.65a	12
	Turkey 1	49ab	29.3bc	483.3a	4.82ab	23.30a	17
2	Iran 1	50ab	34.4abc	632.2a	4.33ab	27.37ab	24
	USA-NE	70ab	51.3ab	854.0a	5.11ab	43.63ab	24
3	Afghanistan 1	70ab	62.2a	876.3a	5.35a	46.88ab	24
	Iran 2	87a	70.2a	1,129.5a	5.39a	60.88b	24
4	Afghanistan 2	84a	62.2a	974.4a	5.21ab	50.76ab	24
	Greece	49ab	38.3abc	527.0a	4.50ab	23.72ab	14
5	USA-MD 1	58ab	43.4abc	790.7a	4.96ab	39.22ab	24
	USA-MD 2	78ab	50.6ab	929.1a	5.07ab	47.11ab	24

Within a column, different letters following least squared means indicate significant differences at α=0.05

comprising genotypes inoculated with repetitions 1 and 2, and run 2 in September/October comprising repetitions 3 and 4. The growth chamber was set at 9 h days with 22 °C day temperature, and 18 °C night temperature. After 7 days, plants were thinned to one plant per unit, and sterile N-free nutrient solution was supplied as needed.

Assessment of symbiotic efficiency

After 6 weeks of growth, plants were harvested, and shoots dried at 65 °C for 24 to 48 h then weighed. Due to low sample mass, shoots were manually ground using mortar and pestle, and samples were analyzed for tissue N by combustion using a PerkinElmer 2400 CHN analyzer (PerkinElmer, USA), where tissue N and biomass then provided an estimate of N fixation. Nodulation was evaluated by measurement of nodule number and nodule mass. Plant roots were harvested to assess nodulation efficiency, with nodule number per plant and total nodule mass per plant recorded. Nodules were then dried in a desiccant, nodules weighed, and total nodule mass per plant recorded.

Determination of resident Rlv *nodD* gene copy numbers

To estimate rhizobia population size and/or nodulation capacity for targeted Rlv strains in each field, *nodD* gene copy numbers were estimated using quantitative PCR (qPCR) approach. Soil samples from triplicate sets of 0.5 g within each treatment were subjected to DNA extraction using FastDNA® Spin Kit for soil (MP Biomedicals, Solon, OH) according to the manufacturer's protocol. The purified salt-free primers (Eurofins MWG Operon, Huntsville, AL) *nodD* F88 and R443 (Macdonald et al. 2011) generate an amplicon of approximately 355 bp in length and were used to amplify the targeted Rlv *nodD* gene. The 10-μl final reaction volume contained 12.5 ng template soil DNA, 2× SsoAdvance™ SYBR® Green Supermix (Bio-Rad), and 300 nM for each forward and reverse primer. A tenfold dilution series of PCR amplified DNA fragments of the *nodD* gene from Rlv strain 3841 (Young et al. 2006) were used to develop qPCR standards. The qPCR was conducted in triplicate for all standard and soil samples in a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad) programmed with the following profile: 98 °C for 2 min, followed by 45 cycles composed by 98 °C for 10 s, 60 °C for 30 s, and 72 °C for 30 s. Amplification specificity was confirmed by melting curve analysis between 60 and 95 °C as well as standard agarose gel electrophoresis. Data were analyzed with the CFX Manager™ Software Version 2.1 (Bio-Rad).

Data analysis

Statistical analyses were performed using SAS ver. 9.2 Statistical Software (SAS, Cary, NC). A combined analysis was performed for both runs. Number of nodules, nodule mass, plant biomass, and biomass N were analyzed using the mixed models procedure (PROC MIXED). A simple t test was used to determine differences among field site pH values. Field history effect was tested with run and all run interactions as random effects. Due to small number of study sites, the model used included site as a fixed effect. All parameters were square root transformed for analysis, and reported least squared means back transformed for data presentation. Mean separations were performed using Tukey's honestly significant difference with α=0.05. The analysis of variance was used to test the effect of HV history on Rlv *nodD* copy numbers using SPSS Statistics ver. 17. A paired t test was performed to test for differences (p<0.01) between HV history within location.

Results

Soil chemical properties

Soil pH $_{(H2O)}$ values were similar among HV+ and HV− fields and were in the range of 6.6 to 5.4 across all sites (Table 2). Soil phosphorus (P) was highest at Ivanhoe and almost ten times lower in Graham. Similarly, the Graham and Cedar Grove copper (Cu) contents were a little over a tenth of those in Ivanhoe HV− field. Notably, manganese (Mn) content was greatest in the Graham HV+ field, 26 % lower in the HV− field, and over 65 % lower in all other fields.

Effect of HV cultivation history on nodulation and symbiotic efficiency

There were significant effects of hairy vetch cultivation history on nodule number and mass, as well as plant biomass and tissue N. Across all sites, HV+ fields had higher nodule number and mass (Fig. 1a, b) than HV− fields. The Ivanhoe HV− field had the poorest nodulation, with a reduction in nodulation of more than 80 % compared to Ivanhoe HV+ field. Averaged across all genotypes, HV− fields from Graham and Cedar Grove had 32 and 35 % reduction in nodule number, respectively, compared to the HV+ fields at those locations. A positive effect of past hairy vetch cultivation on nodule mass was observed in all the three sites. Nodule mass was reduced by 23 % in the HV− field in Graham, with greater reductions of 58 and 73 % in Cedar Grove and Ivanhoe, respectively.

There was a strong site-by-field-history interaction effect on plant biomass ($p=0.0003$) and shoot N ($p<0.0001$). Plants inoculated with soil dilutions from the HV+ field from Ivanhoe had nearly 70 % greater shoot biomass than those inoculated with dilutions from the HV− field. Overall, within a location, genotypes inoculated with soil from fields with HV history had at least 20 % greater shoot biomass than genotypes that were inoculated with soils with no HV history. Plants inoculated with soil dilutions from HV+ fields also had greater mean shoot N concentrations than those inoculated with soil dilutions from HV− fields, with significant differences observed in Cedar Grove and Ivanhoe, but not in Graham. As with nodulation, genotypes inoculated with HV− soils from Graham had over 25 % greater tissue N concentrations than HV− from Cedar Grove and Ivanhoe.

Effect of HV genotype on nodulation and symbiotic efficiency

Hairy vetch genotype had a significant effect on nodule number and nodule mass (Table 3). Group 1 genotypes, Turkey 1 and Turkey 2, had the lowest mean nodule number, 32 and 49 nodules per plant, respectively. The highest mean nodule number was obtained from the Iran 2 genotype, with over 60 % more nodules than the least-nodulated Turkey 1. Moreover, the difference in nodule mass between Iran 2, with the highest mean nodule counts, and Turkey 1, with the lowest mean nodule counts, was over 70 %. The number of nodules found on group 3 genotypes (including Afghanistan 1 and Iran 2 genotypes) was more than three times the number found on group 1.

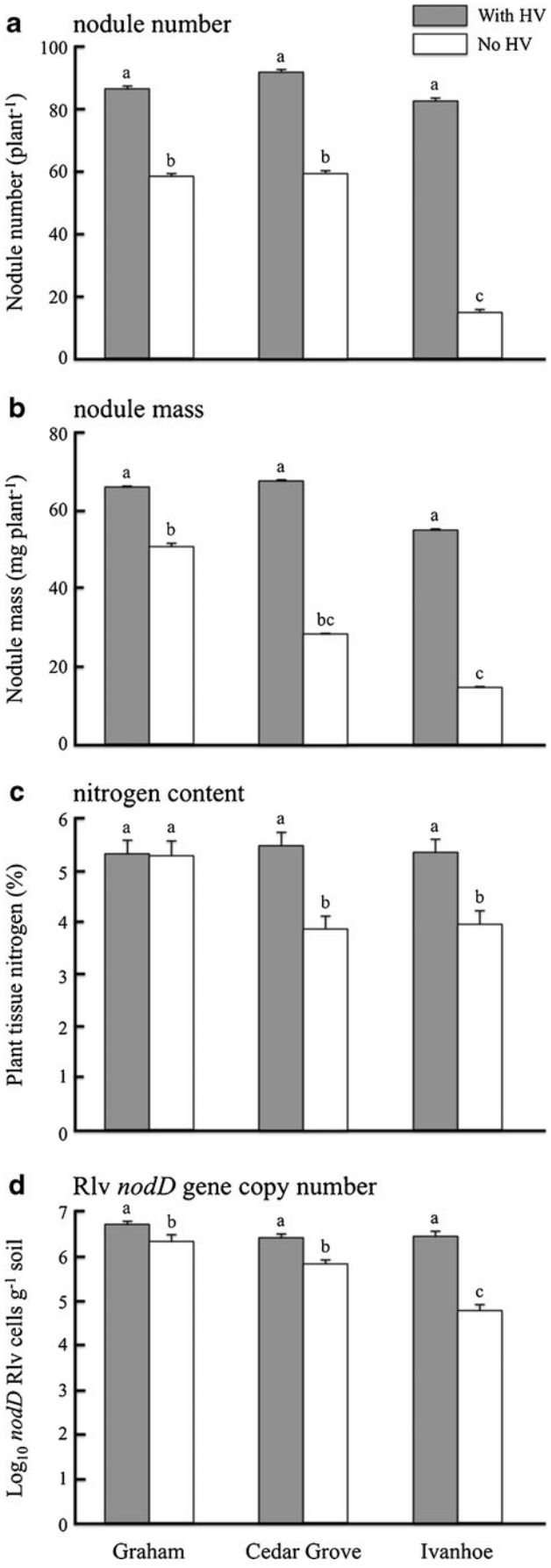
There was no significant genotype effect on biomass production ($p=0.1299$); however, shoot biomass between cultivars varied from a mean of 390 mg plant^{-1} in Turkey 1 to 1,129 mg plant^{-1} in Iran 2; with biomass as low as 20 mg plant^{-1} (Turkey 1) and as high as 2,271 mg plant^{-1} (Iran 2) recorded. Shoot N concentration varied significantly between genotypes (Fig. 1c, $p<0.0001$). As with nodulation and biomass, highest N concentration, 5.38 % was obtained in Iran 2 and lowest N concentration, 4.01 %, in Turkey 1. Overall, group 3 genotypes showed the highest symbiotic efficiency in BNF, containing 20 % more N than group 1 genotypes. The Turkey 2 genotype had high N content, nearly 90 % of the maximum, which is surprising since this genotype was one of the least nodulated with relatively low shoot biomass.

Effect of HV cultivation history on Rlv *nodD* gene copy numbers

In all sites, HV+ fields were found to have significantly larger population sizes of nodulation gene-carrying Rlv (*nodD* Rlv) than those in HV− fields, estimated comparing *nodD* Rlv cell numbers present in HV+ fields to those in HV− fields ($p<0.01$). Using qPCR, the numbers of *nodD* Rlv were estimated using presence of the *nodD* gene based on quantification of *nodD* gene copy numbers in soil samples against the Rlv strain 3841 standard curve, the total amount of DNA extracted from soil, and the amount of DNA template used in the reaction. The standard curve slope was −3.558, an amplification efficiency (E) of 91.0 % with an R^2 of 0.997. Since *nod* genes are located on a single-copy plasmid, qPCR values obtained with the *nodD* primers equal the number of Rlv cells present in the sample (Macdonald et al. 2011), and thus provide an estimation of rhizobia population size. The *nodD* Rlv cell numbers in HV+ fields were quantified at 5.24, 2.63, and 2.84×10^6 cells g^{-1} soil in Graham, Cedar Grove, and Ivanhoe, respectively (Fig. 1d). Cell numbers of *nodD* Rlv in HV− fields were 2.22×10^6, 6.87×10^5, and 6.15×10^4 cells g^{-1} soil, respectively, for the same sites.

Discussion

This study showed that fields where hairy vetch had been cultivated at least five of the past 20 years contained resident populations of rhizobia able to successfully nodulate and fix



◀ **Fig. 1** **a** Effect of hairy vetch cultivation history on nodule number. **b** Effect of hairy vetch cultivation history on nodule mass. **c** Hairy vetch shoot nitrogen content across the three sites. **d** Effect of HV cultivation history on Rlv *nodD* gene copy numbers. *Bars* represent standard errors among all the replicates. *Different letters* indicate significant differences at α=0.05 (**a**–**c**) and 0.01 (**d**)

N with multiple hairy vetch genotypes without additional inoculation, and that *nodD* Rlv populations in fields with a history of hairy vetch planting were larger than fields without. Hairy vetch plants inoculated with soil from HV+ fields were well nodulated, while poor, and in some cases negligible, nodulation was observed in plants inoculated with soil from HV− fields. The results are consistent with previous work (Chemining'wa and Vessey 2006) showing optimal nodulation of pea in fields with pea histories, and poor nodulation in fields never planted to pea. The reduced nodulation ability of these resident pea rhizobia was suggested to be a loss of symbiotic effectiveness over time without the presence of the host legume (Chemining'wa and Vessey 2006). Rhizobia with high saprophytic efficiency, defined by their efficient use of available soil C resources for growth in the absence of a host plant, have been found to have a lower symbiotic efficiency when the host is subsequently introduced into the environment (Duodu et al. 2005). Rhizobia population size also affects host nodulation (Patrick and Lowther 1995), with soils containing larger populations demonstrating enhanced nodulation. Together, these results suggest that despite infective resident rhizobia inhabiting all fields tested in this study, HV− fields had low rhizobial population size or probably contained rhizobial populations with saprophytic efficiency yet low nodulation efficiency. The low nodulation thus appeared to ultimately limit N supplied to host plant.

The Graham HV+ field was unique in that it had never been inoculated with Rlv. Thus, the significantly higher nodulation in the Graham HV+ field and greater *nodD* Rlv cell number when compared to the Graham HV− field indicated a larger nodulation-capable rhizobia population due to previous HV cultivation. However, the lack of differences in total plant N between vetches inoculated with Graham HV+ and HV− soils was curious, and may suggest higher rates of BNF from resident Rlv populations from the Graham HV− field that compensated for the lower nodulation observed across genotypes. High BNF efficiency from resident populations of rhizobia in the HV− field may have resulted from population mixing between the HV+ and HV− fields, as they were in close proximity to each other. Our results provide evidence that in the North Carolina soils evaluated in this study, regular farmer use of hairy vetch as a winter cover crop, with or without inoculation, serves to increase *nodD* Rlv population size and nodulation ability of resident rhizobia, as well as in many cases improves N fixation.

Despite inoculation of the Cedar Grove HV+ field with Rlv each season in which HV was planted, experimental plants inoculated with soil dilutions from this field had similar nodulation to those inoculated with soil from HV+ fields without continual Rlv inoculation. Likewise, Ballard et al. (2004) showed pea inoculation to not significantly increase nodulation in over 20 of 30 tested soils. This lack of differences between recently inoculated and never-inoculated fields suggests that in some cases inoculation benefits may be minimal in these NC systems. Although this study did not assess the effect of inoculation on nodulation, questions remain regarding the need for inoculation in fields with history of HV, and further research on nodule occupancy and soil-resident strain efficiency in BNF is warranted.

All hairy vetch genotypes assessed in this study nodulated when inoculated with soil from the six field sites, indicating that tested soils contain at least minimal populations of infective Rlv needed for nodulation. The differences in nodulation observed between HV− fields of different sites, with some clearly having less effective or smaller populations of resident rhizobia than others (for example, Ivanhoe), suggest impacts of site-specific factors on resident rhizobia populations. In addition to nominally lower pH in the Ivanhoe field without history compared to other HV− sites, this field also had the highest copper content. Rhizobia, particularly Rlv, have been shown to be affected by soil copper content, with high content resulting in reductions in nodule number (Laguerre et al. 2006). Within all three sites, HV− fields had slightly lower pH than the HV+ fields, but differences were not statistically detectable across all sites. Since pH is known to be a driving factor in survival of rhizobia in the field (Andrade et al. 2002a, b; Lapinskas 2007; Hungria and Vargas 2000), lower pH at sites where hairy vetch had never been cultivated may have impacted rhizobia survivability. However, pH levels as those observed here are unlikely to induce conditions that challenge rhizobia survivability (Evans et al. 1980, 1993; Ibekwe et al. 1997). Evidence provided in this study suggests that pH was not a driving factor in nodulation success in the assessed fields. For example, no significant difference in nodulation was observed between the Graham and Ivanhoe fields with hairy vetch cultivation history and sharply contrasting pH values, 6.5 and 5.7, respectively. Further, fields with similar pH values yet contrasting vetch histories, specifically Ivanhoe fields with pH of 5.7 and 5.4, respectively, were found to have significantly different nodule number and mass despite these pH values.

Rhizobia strains resident to soils in North Carolina were able to effectively nodulate hairy vetch genotypes from diverse regions of the world, including Afghanistan, Greece, Iran, and Turkey. Howieson et al. (2005) showed that legume hosts with no history of co-evolution with a particular symbiont have poor nodulation. Strains of *R. leguminosarum* have been shown to share a common phylogenetic origin, and some authors suggest that they have spread trans-continentally in Africa, America, and Asia with *V. sativa* seeds (Alvarez-Martinez et al. 2009). Alvarez-Martinez et al. (2009) further showed *R. leguminosarum* from *V. sativa* isolated from soils in Spain to be phylogenetically related to *V. sativa* rhizobia strains isolated from Africa, America, and Asia. One can therefore speculate that North Carolina resident Rlv strains may be related to those found in the HV centers of diversity of Afghanistan, Greece, Iran, and Turkey, and that through a process of co-evolution with HV, have been introduced to NC along with populations of HV commonly cultivated in the USA.

Symbiotic capacity is defined here as the potential of a legume or rhizobia to nodulate with a partner and result in N fixation. Our results showed variation in symbiotic capacity of distinct HV genotypes with resident North Carolina rhizobia. Cultivar selection within the same legume species has been shown to affect nodulation and BNF in pea (Fettell et al. 1997; Abi-Ghanem et al. 2011), and subterranean clover, with some cultivars being compatible with a wide range of rhizobial strains and resulting in higher BNF (Drew and Ballard 2010). Due to great variation in nitrogen fixing ability of different rhizobial strains, and the difficulty in predicting competitive ability of resident rhizobia, some have suggested that emphasis should be placed on the selection of host genotypes as a means of improving the efficiency of legume-rhizobia symbiosis (Ballard and Charman 2000; Drew and Ballard 2010; Ballard et al. 2002). Our study shows differences in nodulation and total N fixed between populations of hairy vetch. Such genotypes may possibly be able to be used as gene stocks in cover crop breeding programs, improving N fixation ability while developing plants with traits that make hairy vetch more amenable to inclusion as a winter cover crop in farming system niches where specific plant biochemical characteristics are desired. Group 1 genotypes, as well as Iran 1 and Greece, expressed poor nodulation and lower germination rates in all soils examined. The possibility exists that the low nodulation of these genotypes is related to their poor germination and root development rather than ineffectiveness of symbiosis (Barret et al. 2011).

Shoot N concentration results showed that resident rhizobia, particularly those from HV+ fields, are capable of BNF with the host plants evaluated in this study. Although results showed a relatively low correlation of nodule number and shoot biomass, nodule number can be an important predictor of improved legume performance and yield. Sprent et al. (1988, in Voisin et al. 2010) indicated that nodule number plays an important role in host tolerance and adaptation to environment, and nodule mass influences the amount of N fixed. Thus, both nodule number and mass are critical indicators of legume productivity, and mechanisms of how legumes regulate these parameters require further attention.

Conclusions

This study determined the effect of hairy vetch cultivation history on symbiotic efficiency and resident rhizobia population size, and determined how distinct HV genotypes vary in their symbiotic capacity when associating with resident rhizobia. Results suggest that infective strains of Rlv present in North Carolina soils are indeed able to nodulate a diverse array of hairy vetch genotypes. More importantly, decreased nodulation and *nodD* Rlv cell counts in fields without histories of HV compared to fields where hairy vetch had been previously cultivated were observed, suggesting that Rlv population size in soils with no history is generally low and/or that populations have low symbiotic capability. Comparably higher nodulation was found in fields with HV history, supporting our finding that use and history of host legumes enhanced the population size of resident rhizobia and their ability to competently nodulate the host. Variability in symbiotic efficiency of different genotypes provides evidence that BNF efficiency also varies by plant biochemistry. As use of hairy vetch in organic farms across the USA, and globally, continues to increase, knowledge of management practice effects such as continued cultivation of cover crop legumes on nutrient management becomes increasingly critical.

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