8-2022

Reproduction of Soybean Cyst Nematode Populations on Field Pennycress, Henbit, and Purple Deadnettle Weed Hosts

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agronomy

MDPI

Communication

Reproduction of Soybean Cyst Nematode Populations on Field Pennycress, Henbit, and Purple Deadnettle Weed Hosts

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Abstract: Several weeds serve as alternative soybean cyst nematode (SCN) hosts. Still, the relative reproductive capacity of SCN HG types (*Heterodera glycines* type) on weed hosts relative to soybean is not well understood. This study examined the reproduction of three South Dakota endemic SCN populations—PSCN-1 (HG 0), PSCN-2 (HG 2.5.7), and PSCN-3 (HG 7)—on purple deadnettle, field pennycress, and henbit. The Relative Female Index (RFI) was calculated to compare SCN reproduction relative to the susceptible soybean check. Weed hosts, HG types, and their interactions influenced SCN reproduction. Henbit (RFI = 51.8) and purple deadnettle (RFI = 47.6) roots had a similar high RFI, whereas field pennycress (RFI = 23.04) had a lower RFI. Similarly, SCN populations PSCN-1 and PSCN-3 had a similar RFI of 36.9 and 37.2, respectively, while the population PSCN-2 had a higher RFI of 44.9 across weed hosts. A significant interaction between PSCN-1 and purple deadnettle was observed where the RFI was the highest (RFI = 53.3). These results indicate that these weed hosts support endemic SCN populations, and the HG type influenced reproductive success, further complicating SCN management. Hence, SCN presents a significant challenge in the new prospect of incorporating field pennycress host as an oilseed cover crop in the Midwest's corn–soybean production system.

Keywords: *Heterodera glycines*; HG types; soybean cyst nematode; relative female index; disease management; field pennycress

Citation: Basnet, P.; Clay, S.A.; Byamukama, E. Reproduction of Soybean Cyst Nematode Populations on Field Pennycress, Henbit, and Purple Deadnettle Weed Hosts. *Agronomy* **2022**, *12*, 2027. https://doi.org/10.3390/agronomy12092027

Academic Editors: Ivan Hiltpold, Sergio Rasmann, Andrea C. Ruthes and Paul Dahlin

Received: 20 July 2022
Accepted: 23 August 2022
Published: 26 August 2022

1. Introduction

Several biotic and abiotic constraints affect soybean (*Glycine max* (L.) Merr.) production in the United States. Among the biological constraints, soybean cyst nematode (SCN; *Heterodera glycines*, Ichinohe) is the most critical yield-limiting factor [1–3]. SCN is estimated to cause a USD 1.5 billion loss of revenue in the United States, making it the most devastating soybean pathogen [2–5]. In South Dakota, SCN has been reported in 34 soybean-producing counties, causing yield loss estimated at 0.12 million metric tons annually [6,7].

Soybean cyst nematode is an obligate and sedentary endoparasitic cyst-forming nematode that causes chlorosis, premature defoliation, stunting, and root damage, which lead to severe yield loss [8]. The accrued average soybean yield reduction is estimated at over 60% [2]. SCN management is complicated and challenging because no in-season management strategies are currently available [9]. Using SCN-resistant cultivars and crop rotation with non-host crops are common SCN management practices [10–13]. However, the presence of SCN weed hosts can minimize the effectiveness of host resistance and crop rotation by sustaining continued SCN reproduction in the field [14–16].

Winter annual weeds play an important role in the biology of the plant parasitic nematodes [17]. The SCN life cycle starts with the fertilized eggs, which change to the infective second stage juvenile capable of finding roots and feeding [9]. Typically, SCN takes around 3 to 4 weeks to complete its life cycle, which is influenced by several environmental factors [9,18]. Depending on the soybean maturity group planted in South

Dakota, SCN can complete up to 3–6 life cycles in a single growing season [19,20]. The presence of weed hosts facilitates the completion of the SCN life cycle in the absence of a primary soybean host and increases SCN population density [20]. Several studies have been conducted to evaluate alternative SCN weed hosts and 116 SCN weed hosts have been determined through field surveys and greenhouse studies [21]. The most commonly known alternative weed hosts include purple deadnettle (*Lamium purpureum* L.) [16,22,23], henbit (*Lamium amplexicaule* L.) [16,22,24,25], and field pennycress (*Thlaspi arvense* L.) [16,23,26,27]. Understanding SCN adaptability on weed hosts will be crucial for designing sustainable SCN management strategies, including proactive management of alternative weed hosts in the corn–soybean crop production system [16,21,23]. However, different biotypes of weed host species, the complexity in SCN populations, and selection pressures associated with the continuous use of resistant cultivars present significant challenges [10,12,13].

Determining the reproductive capacity of SCN populations on weed hosts is essential in understanding their influence on field SCN population density. The weed host's green bridge harbors the SCN population in the absence of primary soybean hosts [22,23,28]. With recent efforts in incorporating field pennycress as an oilseed cover crop in the Midwest's corn–soybean production system, understanding weed hosts–SCN pathosystem—particularly in field pennycress—becomes more crucial [23,29,30]. Only a few studies have evaluated alternative weed hosts using SCN populations with different HG types. The weed species were mainly evaluated with SCN HG type 0 (race 3), the predominant SCN population type in the United States [16,27]. With the continuous use of the predominant PI 88788 resistance source, SCN populations with HG type 0 have adapted to the resistance source, altering the virulence profiles of the SCN populations in the soybean growing regions [12,31–33]. Since the shifting of field SCN populations towards more virulent SCN populations (HG type 2.5.7 and 1.2.5.7) are increasing due to selection pressure, there is a need to understand the reproduction of SCN populations on common SCN weed hosts. Hence, the objective of this study was to determine the reproduction of three common endemic SCN field populations on three primary weed hosts—purple deadnettle, field pennycress, and henbit—under greenhouse conditions.

2. Materials and Methods

2.1. Source of SCN Inoculum

This study used three South Dakota endemic SCN populations—namely, PSCN-1, PSCN-2, and PSCN-3—as a source of inoculum. These populations were collected from SCN-infested fields as reported [34]. SCN populations were increased on the susceptible Williams 82 cultivar in the greenhouse, and HG type test was conducted to confirm their population types. Four replicates of pre-germinated seeds of indicator lines (Peking, PI 88788, PI 90763, PI 437654, PI 89772, and Cloud) and a susceptible cultivar (Williams 82) were transplanted into individual cone-tainer (3.8 cm diameter and 21 cm height, Stuewe and Sons Inc., Tangent, OR, USA) placed in plastic buckets and filled with sterilized soil mixture (2 parts of sand and 1 part of soil by volume). The soybean seedlings were inoculated with 1000 eggs in a pencil hole 6 cm below the soil surface. The buckets were then placed in a water bath maintained at the temperature of 28 °C and daylight length of 16 h in a greenhouse. After 30 days post-inoculation, cysts from each plant were collected in 210 µM pore sized sieves nested under 710 µM pore sized sieves using a strong stream of water and counted in an inverted microscope. HG types for these populations were reconfirmed in the greenhouse study as 0, 2.5.7, and 7, respectively (Table 1).

Table 1. Female index (FI) of *Heterodera glycines* populations used in the study.

SCN Population	SCN FI on Indicator Lines (%)							Williams 82 (Check)	HG Type
	1 Peking	2 PI 88788	3 PI 90736	4 PI 437654	5 PI 209332	6 PI 89722	7 Cloud		
PSCN-1	1.1	1.8	0.6	0.04	1.7	0.7	4.5	210	0
PSCN-2	3.2	21.1	0.9	0.1	16.4	1.5	28.8	210	2.5.7
PSCN-3	1.8	3.8	0.4	0.1	3.6	0.8	14.7	221	7

SCN HG type is determined by ≥10% reproduction on a PI line relative to the susceptible check (number of cysts on the PI line/number of cysts on the susceptible check × 100).

2.2. Experiment Setup

Three common SCN weed hosts—purple deadnettle, field pennycress, and henbit—were used in this study. The weed species and the susceptible soybean cultivar Williams 82 (check) were transplanted 3 to 5 days after germination. The SCN populations were increased in the greenhouse by using the susceptible soybean cultivar Williams 82. Cysts obtained from Williams 82 were used to prepare inoculum following the standard SCN eggs and juvenile extraction procedure [35]. The eggs and juveniles were suspended in distilled water at a concentration of 1000 eggs and juveniles per mL, and a 2 mL inoculation volume was used for each treatment. Plants were transplanted individually into cone-tainers (3.8 cm diameter and 21 cm height, Stuewe and Sons Inc., Tangent, OR, USA) filled with sterilized soil (2 parts sand and 1 part clay soil by volume). The bottom of each cone was tied with a double-layered weed barrier to prevent the leakage of SCN inoculum. After transplantation, 2 mL volume was used to inoculate each cone-tainer using a plastic transfer pipette by making a pencil hole (6 cm depth). Cone-tainers were then transferred to an 18.9 Lsand bucket (Runnings Inc, Marshall, MN, USA) and placed in a water bath maintained at 28 °C throughout the day and night with supplemental lighting to maintain a daylight length of 16 h. Each replicate consisted of three Williams 82 control and three weed species (one seedling per cone) inoculated with three SCN populations. Thus, altogether a replicate (bucket) consisted of 12 cone-tainers. The treatments were arranged in a completely randomized design with six replications, and the experiment was repeated twice.

2.3. Data Collection

After 35 days, cone-tainers were removed from the buckets and soaked in water for 20 min. The plants were gently removed from the cone-tainers, and roots were washed to collect cysts. Cysts were collected in a 210 µM pore-sized sieve nested under a 710 µM pore-sized sieve by spraying roots with a strong stream of water, and the collected cysts were counted on a stereomicroscope [36]. Six cysts from each treatment were randomly selected to determine the number of eggs per cyst across treatments. A 2 mL plastic transfer pipette was used to place the cysts on a glass slide in a drop of water, and a teasing needle was used to rupture the cysts. Eggs from each cyst were counted using a hemocytometer under an inverted microscope (Nikon Instruments Inc., Melville, NY, USA).

HG type test is widely used to characterize SCN populations based on their reproduction on seven soybean indicator lines [37]. This HG-type concept was introduced to denote specific SCN populations as it is almost impossible to genetically characterize every single nematode from an SCN population, unlike other pathogens [9,36,38]. The female index (FI) is used to express SCN reproductive capacity relative to a susceptible soybean check [37]. However, FI in this study was used to compare SCN reproduction on weed host relative to the susceptible soybean check. Hence, the term relative female index (RFI) was used instead of FI for the comparison. The number of cysts on weed hosts relative to the susceptible soybean check was used to obtain the relative female index (RFI) using the formula

$$\text{Relative female index (RFI)} = \frac{\textbf{Average number of cysts on the weed host}}{\textbf{Average number of cysts on the susceptible soybean check}} \times 100$$

2.4. Data Analysis

The number of cysts that developed on the root, RFI, and the number of eggs per cyst was subjected to analysis of variance using R studio version 3.4.3 (R Core Team, The R Foundation for Statistical Computing Platform, Vienna, Austria). The number of cysts was square-root transformed to normalize variance before performing an analysis of variance. Data were pooled across the two runs after performing Bartlett's homogeneity test [39]. Analysis of variance was used to test the main and interaction effects of HG types and weed species on SCN reproduction. Tukey HSD test for multiple pairwise comparisons was used to separate means at $p \leq 0.05$ using the R package 'Agricolae' [40].

3. Results and Discussion

3.1. Effects of Weed Species and SCN Population on Cyst Counts

The susceptible soybean check (primary SCN host) had the highest number of cysts for all the three SCN populations (Figure 1). However, considerable cyst development was also observed on field pennycress, henbit, and purple deadnettle (Figure 1).

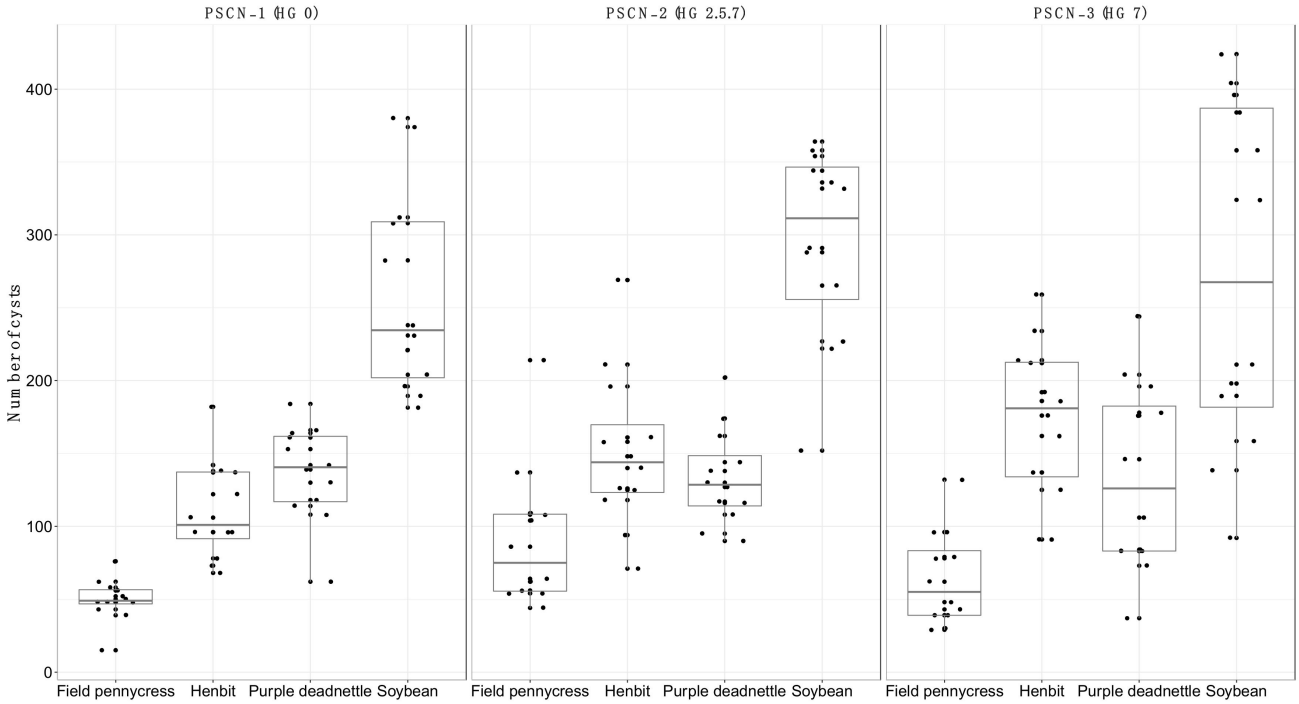

Figure 1. Boxplot showing the number of cysts on three weed hosts (field pennycress, henbit, and purple deadnettle) and the susceptible soybean check across three SCN populations (HG 0, 2.5.7, and 7).

Weed species and SCN population type significantly affected the number of SCN cysts that developed (p-value = 1×10^{-4}; Tables 2 and 3). An interaction between the SCN populations and weed species for cyst counts was also significant (p-value = 0.02; Table 2). Among the weed species, henbit (Cysts = 139.2) and purple deadnettle (Cysts = 130) had similar and significantly higher SCN cyst counts, whereas field pennycress had fewer cyst counts (Cysts = 64) (Table 3). A significant interaction between PSCN-1 and purple deadnettle was observed where the number of cysts was highest among all the three weeds (Tables 2 and 3).

Table 2. Analysis of variance of the effect of weed species and soybean cyst nematode (SCN) populations on SCN cysts, relative female index, and eggs per cyst under greenhouse conditions.

Source	Df	*p*-Value		
		SCN Cysts	**Relative Female Index (RFI)**	**No. of Eggs per Cyst**
Weed species	2	1×10^{-4}	1×10^{-4}	1×10^{-4}
SCN populations	2	1×10^{-4}	0.04	0.71
Weed species × SCN populations	4	0.02	0.01	0.06

Df = degree of freedom; *p*-value ≤ 0.05 indicates significance; Weed species include purple deadnettle (*Lamium purpureum* L.), henbit (*Lamium amplexicaule* L.), and field pennycress (*Thalpsi arvense* L.); SCN populations include PSCN-1 (HG type 0), PSCN-2 (HG type 2.5.7), and PSCN-3 (HG type 7).

Table 3. Effect of weed species and soybean cyst nematode (SCN) populations on cysts development under greenhouse conditions.

Weed Species/SCN Populations	Mean Cysts within Each Weed Species and SCN Populations			Mean Cysts across Weed Hosts
	PSCN-1	**PSCN-2**	**PSCN-3**	
Henbit	108.2 b	174.2 a	141.6 ab	139.2 A
Purple deadnettle	134.6 ab	148.8 ab	110.3 b	130.0 A
Field pennycress	47.6 c	102.0 b	46.7 c	64.0 B
Mean cysts across SCN populations	92.2 B	139.2 A	94.1 B	

The values are pooled mean from six replications and two repetitions after the homogeneity test using Bartlett's homogeneity test. Values followed by the same lowercase letters (representing interactions) are not significantly different according to the Tukey HSD test at $p \leq 0.05$. Values followed by the same uppercase letters (main effects) are not significantly different across rows for mean cysts across weed hosts and columns for mean cysts across SCN populations according to the Tukey HSD test at $p \leq 0.05$.

Among SCN populations, PSCN-3 and PSCN-1 produced similar but fewer cysts on the three weed species, whereas PSCN-2 had a higher cysts number (Table 3). Although there is no absolute measurement of virulence among the SCN HG types, FI has been commonly used by the SCN community as a proxy for aggressiveness and is hence referred to as the "aggressiveness index" [41]. Our results indicate that SCN population with HG type 2.5.7 was more aggressive on the three weed hosts than the other SCN populations. This result is in congruence with previous studies, which had reported HG type 2.5.7 to have higher reproduction rates on the SCN soybean indicator lines [31,34].

3.2. Effect of Weed Species and SCN Field Population on the Relative Female Index

Weed species and SCN population affected the RFI (*p*-value = 1×10^{-4}, and *p*-value = 0.04) (Table 2). The interaction between the SCN population and weed species was also significant (*p*-value = 0.01; Tables 2 and 4). Among weed species, henbit and purple deadnettle had similar RFI across three SCN populations, whereas field pennycress had a lower RFI compared to the other two weed species (Table 4).

RFI for the three SCN populations was similar on the henbit host (Table 4). Our results on SCN reproduction on henbit are within the range of what has been reported in previous studies. A study reported an RFI of 45.5 on henbit for an SCN population HG 0 with an inoculum of 2000 eggs [16], 63.2 on henbit for SCN population HG 2.5.7 [42], and 41.9 on henbit for SCN population HG 1.7 [43]. Hence, results from our studies confirm that henbit is a strong host of SCN for different SCN virulent populations. The only discrepancy from previous studies lies where no cyst development was reported on henbit for HG type 2.5.7 [27]. This might be attributed to differences in collections of henbit accession biotypes and low inoculum levels used [16,23].

Table 4. Effect of weed species and soybean cyst nematode (SCN) populations on the female index under greenhouse conditions.

Weed Species	Mean RFI within Each SCN Population			Mean RFI across Weed Species
	PSCN-1	PSCN-2	PSCN-3	
Henbit	43.56 a	56.6 a	55.7 ab	51.8 A
Purple deadnettle	53.3 a	50.4 ab	41.0 ab	47.6 A
Field pennycress	18.5 c	33.6 bc	16.0 c	23.0 B
Mean RFI across HG types	36.9 B	44.9 A	37.2 B	

The values are pooled mean from six replications and two repetitions after the homogeneity test using Bartlett's homogeneity test. Values followed by the same lowercase letters (representing interactions) are not significantly different according to the Tukey HSD test at $p \leq 0.05$. Values followed by the same uppercase letters (main effects) are not significantly different across rows for mean RFI across weed hosts and columns for mean RFI across SCN populations according to the Tukey HSD test at $p \leq 0.05$. The female index is the proportion of cysts that developed on the weed species relative to the susceptible check, Williams 82.

The SCN populations PSCN-1, PSCN-2, and PSCN-3 had 135, 149, and 110 cysts on purple deadnettle, respectively; and RFI of 53.3, 41.0, and 50.4, respectively. A significant interaction was observed between PSCN-1 and purple deadnettle weed host (RFI = 53.3; Table 4). In a previous greenhouse study, 156 cysts were observed on purple deadnettle for SCN population HG 2.5.7 [44]. A research study also reported mean cysts of 510 and 385 on purple deadnettle for SCN populations with HG types 0 and 2.5.7, respectively [27]. Both the research studies mentioned above reported a higher cysts reproduction on purple deadnettle compared to this study. These inconsistencies could be attributed to using different accession biotypes, SCN populations, SCN population fitness, and inoculum levels. Nonetheless, it suggests that purple deadnettle is a good host of SCN as the SCN phenotype rating mostly fell within the moderately susceptible to susceptible category (RFI > 30) [37].

RFI on field pennycress for the three SCN populations—PSCN-1, PSCN-2, and PSCN-3—were 18.5, 33.6, and 16, respectively. This reproduction of PSCN-1 on field pennycress is consistent with a greenhouse study which reported an SCN RFI of 34 for the SCN population (HG type 0) on field pennycress [16]. Similarly, a greenhouse screening of field pennycress accession with SCN population HG type 0 (density: 4000 eggs per mL per plant) reported variable RFI on different field pennycress accessions ranging from 27 to 143 [30]. However, few studies did not observe the reproduction of the SCN population (HG 2.5.7) on field pennycress [27], as opposed to this study, which is perhaps due to differences in pennycress biotype, SCN populations, inoculum levels, and inoculation methods [16,23]. Although there are inconsistencies with the RFI in field pennycress, most studies concluded that field pennycress is a good host of SCN, and the SCN population type influenced the level of SCN reproduction. This implies that if field pennycress is used as an alternative cover crop, SCN-resistant soybean varieties should be selected, and these crops should not be grown in consecutive years.

3.3. Effect of Weed Species and SCN Populations on the Eggs per Cyst

Weed species significantly affected the number of eggs per cyst across different SCN populations (p-value = 1×10^{-4}; Tables 2 and 5). However, the interaction between SCN population and weed species was insignificant (Table 5). The lack of significant differences in the number of eggs per cyst among SCN populations and weed species is congruent with previous reports. Similar levels of eggs per cyst on various cover crops for an SCN population with HG type 0 were observed [45]. However, in other cyst-forming nematodes, such as potato cyst nematode (*Globodera pallida*), the number of eggs per cyst was lower in a resistant variety compared to the susceptible variety [46]. The variation in the number of eggs per cyst may be related to the nutrient availability and the balance of nutrients [47], but this needs further research.

Table 5. Number of soybean cyst nematode (SCN) eggs per cyst collected from field pennycress, henbit, purple deadnettle, and susceptible check soybean cultivar.

Weed Species	Mean Egg Counts within Each Cyst among SCN Populations			Mean Egg Counts per Cyst across Weed Species
	PSCN-1	PSCN-2	PSCN-3	
Soybean check	456.7 ab	334.0 abc	470.0 a	420.2 A
Henbit	394.0 abc	390.0 abc	396.7 c	393.6 A
Purple deadnettle	369.2 abc	385.7 abc	317.2 abc	357.3 AB
Field pennycress	293.8 c	330.8 abc	309.5 bc	311.4 B
Mean egg counts per cyst across SCN populations	378.4 A	360.1 A	373.3 A	

The values are pooled mean from six replications and two repetitions after the homogeneity test using Bartlett's homogeneity test. Values followed by the same lowercase letters (representing interactions) are not significantly different according to the Tukey HSD test at $p \leq 0.05$. Values followed by the same uppercase letters (main effects) are not significantly different across rows for mean eggs per cyst across plant species and columns for mean eggs per cyst across SCN populations according to the Tukey HSD test at $p \leq 0.05$.

Winter annual weeds usually emerge during late fall, overwinter during the winter, and complete the life cycle during spring [48]. The germination and emergence of SCN weed hosts affect the level of SCN infection during the late fall and early spring [21,23,44]. Several abiotic factors—including soil temperature, moisture, and light quality—further affect the germination and emergence of winter annual weed hosts [49]. It has been suggested that the ability of SCN to infect the winter annual weeds hosts lowers significantly when the temperature falls below 10 °C [26]. A field research study had shown that field pennycress had the second-highest emergence (i.e., 27%) across different locations in Nebraska [50]. Since our study mainly focused on understanding the impact of the SCN population on primary weed hosts in greenhouse conditions, we did not consider these factors. In addition, different weed host biotypes have been shown to support the SCN population differently [16,23]. On the contrary, SCN was found to survive and develop in purple deadnettle during the dormant stage under cold conditions [51]. This indicates that the fall emergence of the winter annual weed hosts is critical in influencing SCN population levels in the soil [51]. However, the ability of SCN to survive on the overwintering weed hosts during winter is not well studied and warrants further research. Additionally, there have been limited research studies to understand the reproductive ability of SCN on weed hosts during the fall and winter. A study reported a significant number of cysts development on winter annual weed hosts during fall than winter [44]. Although temperature during winter is not optimal for SCN development, SCN can reproduce and develop on winter annual weed hosts in the absence of crops [22,51]. This implies that proactive management of winter annual weed hosts should be carried out during fall and early spring to prevent SCN population buildup in the field. The continuous presence of SCN weed hosts can influence SCN adaptability to different sources of resistance, as indicated by the differential reproduction of the three SCN populations on the three primary weed hosts in this study. A recent resistance rotation study reported that continuous planting of a susceptible soybean variety for 12 years reduced SCN virulence to the Peking source of resistance [52]. Although no information is available on the resistance genes in weeds against SCN, continuous reproduction of SCN in these weeds could influence their SCN virulence. Thus, the SCN weed hosts can affect SCN population density and HG types.

A previous study to determine the weed hosts' abundance in soybean fields in South Dakota had shown that field pennycress was found in more than 50% of the sampled areas, whereas purple deadnettle was found only in 4% of the sampled fields [23]. Although reproduction of the three SCN populations on field pennycress was slightly lower in this study, its significant abundance and tremendous field emergence potential in South Dakota make it a critical SCN weed host [23,51]. Recent research on the reproduction of SCN populations on pennycress determined pennycress to be an alternative SCN host in the

field and the greenhouse conditions, with the potential to impact field SCN population density [53]. Besides, field pennycress is being extensively studied for its domestication and adoption as the winter annual oilseed and cover crop in the corn–soybean production system [29]. However, the ability of SCN to infect and reproduce on pennycress could be a significant challenge for both pennycress and soybean growers [23]. With SCN already being a major soybean pathogen, causing an annual loss of USD 1.5 billion, pennycress inclusion as an annual winter crop will facilitate the buildup of the SCN population in the field [20,23]. Because the SCN population with HG type 2.5.7 had a significant cyst development on field pennycress, this host could favor the SCN population with a diverse virulence profile, ultimately leading to accumulation of aggressive SCN populations in the field. Our findings suggest that proactive SCN weed host management in the fall and early spring should be conducted to limit the development of aggressive SCN populations and minimize SCN inoculum buildup in the field.

Author Contributions: Conceptualization, methodology, investigation, formal analysis, writing, original draft, reviewing, and editing, P.B.; conceptualization, writing, reviewing, and editing, S.A.C.; Conceptualization, methodology, investigation, reviewing, editing, funding acquisition, E.B. All authors have read and agreed to the published version of the manuscript.

Funding: Financial funding was received from South Dakota Soybean Research and Promotional Council and USDA NIFA Hatch grant no. SD00H662-18.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

Acknowledgments: We also acknowledge help from Paul Okello, Rawnaq Choudhury, and Krishna Acharya for their valuable suggestions for this project. We thank Richard Geppert, Dalitso Yabwalo, and Connie Tande for their assistance in various aspects of the study.

Conflicts of Interest: The authors declare no conflict of interest.

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