22

Soybean Cyst Nematode (*Heterodera glycines* Ichinohe)

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1. Introduction

Soybean cyst nematode (SCN) (*Heterodera glycines* Ichinohe) is the most serious nematode pest on soybean in the world, infests most of the soybean producing countries of the world with the exception of west European countries and Oceania countries and causes up to 1.5 billions US$ economical loss according to some estimates (Wrather et al., 2001). The cyst nematode is also an international quarantined pest. Although it was first discovered and described in Japan in 1952, it is now widely believed originated in China as its host soybean was. In America, since the first report in North Carolina, USA in 1955, it has spread to 26 out of 28 soybean producing states, to the province of Ontario, Canada, and to the soybean producing countries of South America. Race of the nematode was recognized in 1954, and a total of 14 races were reported and widely distributed, especially in the USA, which has created a series of problems for developing resistant cultivars. As the climate change intensifies, it is likely that this nematode pest is going to spread to new soybean producing areas. Many resistant cultivars have been developed, especially in USA where resistant cultivars were developed using resistant parents selected from resistant plant introductions (PI) of the exotic accessions in the USDA soybean germplasm collection. These resistant PIs were collected from the oriental countries. Since the 80s, international seed companies like Pioneer, and Monsanto have been the driving force for the resistant cultivar development and marketing. SCN resistant cultivars alone used to be the solution for the control of the nematode pest for soybean production in USA. Because of the new emerging races and the shifting between existing races, resistant cultivars in many cases lose their usefulness dramatically. With the new realization that the agriculture biodiversity plays an essential role for pest management, new control methods have been developed and tested such as rotating with nonhost crops, planting multiline cultivars mixtures, using biological control agents, and applying green manure. There are a lot of literatures related with SCN, one book by Schmitt et al, 2004, and a review by Noel 1993 nevertheless are the excellent sources of information. A few non scientific aspects are also used for the synthesis of this paper. China, where the nematode is believed originated, becomes economically integrated into the world system at a pace never seen before. Soybeans there as a crop are shifting from vegetable to pulse, and to oil seeds. Soybean seeds are increasingly produced, controlled and marketed by a few international companies, as the result, fewer cultivars (more monocultures) are planted at a given year compared with the time when farmers used to get their seeds from all sorts of channels, and productions are in large scales. The climate change, especially the global warming caused by human activities is inevitably impacting the soybean production, and the soybean cyst nematode.

2. Taxonomic position

When the SCN was discovered, it was believed as a race of *Heterodera schachtii,* the sugar beet cyst nematode, since these 2 species are closely related biologically. Around that time, cyst nematodes were generally considered races of *H. schachtii*. In 1940 Franklin's comparative morphological studies led to many "races" being elevated to species. It was morphologically consistent that the morphological distinctions of the SCN led Ichinohe to elevate the race to a new species in 1952.

Phylum Nematoda,
- Class Secernentea,
  - Order Tylenchida,
    - Suborder Hoplolaimina,
      - Superfamily Hoplolaimoidea,
        - Family Heteroderidae,
          - Genus *Heterodera*,
            - Species *Heterodera glycines* (Ichinohe 1952)

3. Morphology and identification

H. glycines is a typical cyst forming nematode within the family of Heteroderidae: characterized by sexual dimorphism: male is vermiform, while female lemon shaped (Figure 1). The brown cyst is the dead female with viable eggs inside. The second stage juvenile (J2) is vermiform, much smaller than the male. The length of the stylet, and the hyaline tail terminus of J2, and the characters of the vulva cone of the cyst are the most important characters for the identification (Table 1). More than one reference descriptions are usually required for comparison because there are variance among isolates from different crops and locations (Mulvey & Golden 1983, Wouts 1985, Golden 1986, Burrows & Stone 1985, Tylor 1975, Hesling 1978, Graney & Miller 1986).

Fig. 1. Morphology of *Heteridera glycines*: A: male; B: J2; C: head of J2; D: cyst; E: hyaline tail terminus

Character		Measurment (µ)	
Cyst		**Average**	**Range**
Fenestra	**length**	**55**	**30-70**
	width	**42**	**25-60**
Vulval slit	**length**	**53**	**43-60**
J2			
Body	**length**	**440**	**375-540**
Stylet	**length**	**23**	**22-24**
Tail	**length**	**50**	**40-61**
	hyaline length	**27**	**20-30**

Table 1. Measurement of *Heterodera glycines* (after Tylor, 1975, Graney & Miller, 1982)

4. Biology and life cycle

After the death of the female, the eggs are retained inside the hardened body (cyst), until suitable conditions arrive. The cysts can remain viable for several years in the soil. The eggs hatch to juveniles at stage 2, stimulated by exudates from the roots (Masamune et al., 1982). The 2nd stage juveniles (J2) of *H. glycines* are the only stage that the nematode can penetrate the root near the tip. The J2 once inside the root, become sedentary and establish a syncytic feeding site (Moore, 1984). J2 swells, and moults to J3, J4, and become adults (Wyss and Zunke, 1992). The life cycle is usually from 21 to 24 days (Fig. 2). Time required for the nematode to complete its life cycle is usually from 20-25 days at 20-24 ^{0}C, the lower the temperature is, the longer the time it takes to finish its life cycle (Melton et al., 1986).

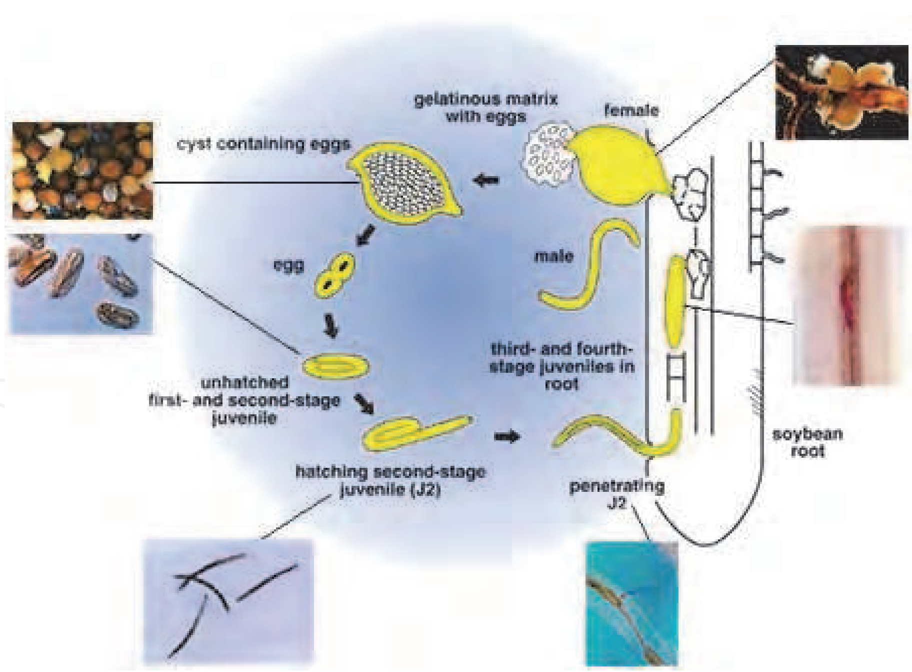

Fig. 2. Life cycle of *Heterodera glycines* (courtesy of Dirk Charlson, Iowa State University)

5. Distribution

It is impossible to know exactly the source(s) of infestation of the nematode species. More and more people believe that *H. glycines* is very likely a native of China, for two compelling evidences: 1. One of the most important hosts for the nematode the soybean originated (domesticated 5,000 years ago, the early stage of the Chinese civilization) (Qiu et al., 2011, Liu et al., 1997) in China, and 2. Most of the resistant cultivars used today have their roots in cultivars from China (Bernard et al., 1988). Probably the spreading and the pathway of the nematode followed footsteps of its host soybean (Fig. 3). The soybean was introduced to Japan, Korea around 300 AD, the nematode was discovered in 1915 (Hori), and later was described by Ichinohe in 1952 with the type locality in Hokkaido. It was first found in the United States in 1954 (Winstead et al., 1955) and spread with the expansion of soybean growing areas such as in Canada (Anderson et al., 1988). The nematode was also found in Colombia in the 1980s, and more recently in the major soybean producing areas in Argentina and Brazil (Mendes & Dickson 1992). SCN has also been reported from Iran and Italy (Fig. 3).

Africa: Egypt (unconfirmed)

Asia: China (Anhui, Hebei, Hubei, Heilongjiang, Henan, Inner Mongolia, Jiangsu, Jilin, Liaoning, Shanxi, Shandong), Indonesia (Java), Korean peninsula, Japan, Taiwan (unconfirmed), Russia (Amur District in the Far East).

North America: Canada (Ontario), USA (Alabama, Arkansas, Delaware, Florida, Georgia, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Maryland, Minnesota, Michigan, Mississippi, Missouri, Nebraska, New Jersey, North Carolina, North Dakota, Ohio, Oklahoma, Pennsylvania, South Carolina, South Dakota, Tennessee, Texas, Virginia and Wisconsin).

South America: Argentina, Brazil, Chile, Columbia, Ecuador.

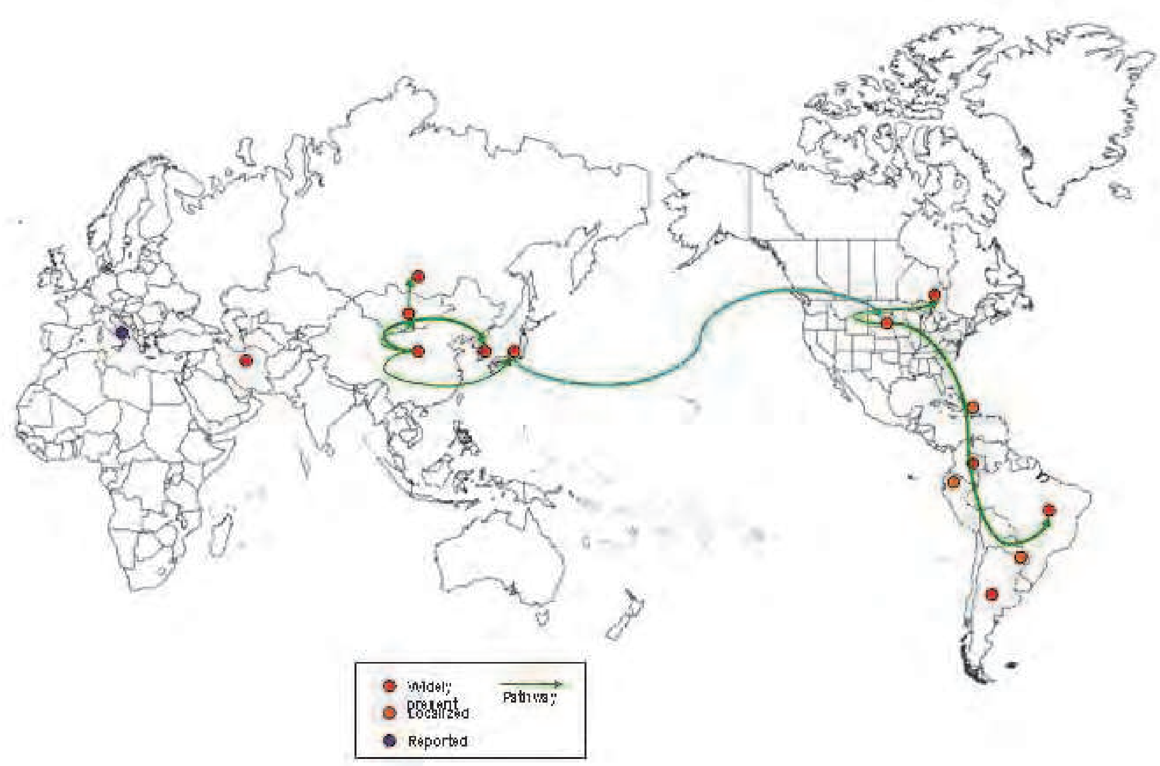

Fig. 3. Distrubition map of *Heterodera glycines* in the World.

6. Disease symptoms

"Yellow dwarf" is a good description of the above ground symptoms when soybeans are infested with the soybean cyst nematode. When soybeans are heavily infested, the plants usually become stunt (Fig. 4). Low level infestation usually does not produce obvious symptoms above ground. Belowground symptoms include poorly developed and darkened roots, reduced nodule formation.

Fig. 4. Above ground symptoms of soybeans infested by soybean cyst nematode

7. Spread

7.1 Canada

Since it was first identified in 1987, SCN has been identified in 12 counties in Ontario, Canada. Infected counties include Essex, Kent, Lambton, Elgin, Perth, Haldimand-Norfolk, Middlesex, Glengarry, Prescott, Stormont, Huron and Oxford. It is obvious that *H. glycines* has been spreading north and northeast wards (Fig. 5). It would be difficult to exclude the possibility that climate change is the cause, or at least one of the causes. This finding proves what Boland et al. 2004 has predicated. It is likely to spread along the St. Lawrence seaway towards the Maritime provinces as new cultivars suitable for the cold climate being developed and planted in the region.

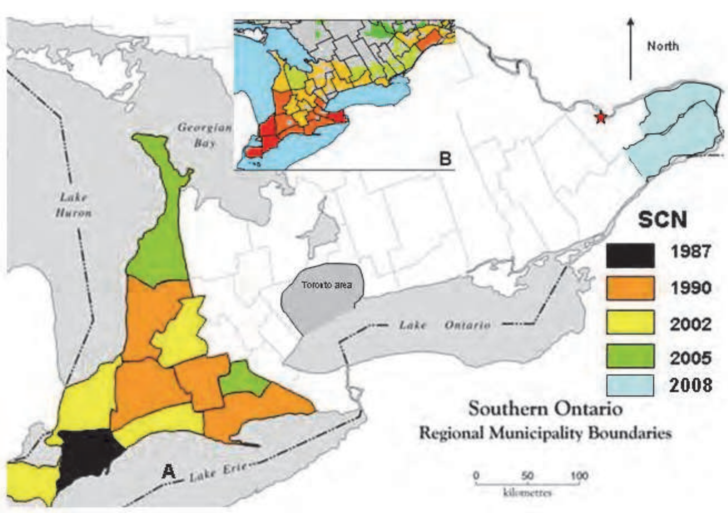

Fig. 5. Distribution of *Heterodera glycines* in Ontario, Canada from 1987 to 2007 (insert B: soybean growing area in Ontario).

7.2 USA

Since *H. glycines* was first discovered in North Caroline in 1955. Within the next 6 years, it had been reported in 7 states along the Mississippi river (Riggs, 2004). Today, it has spread to 29 soybean producing states in USA, as far northeast as the state of New Jersey (Riggs, 2004) (Fig. 6).

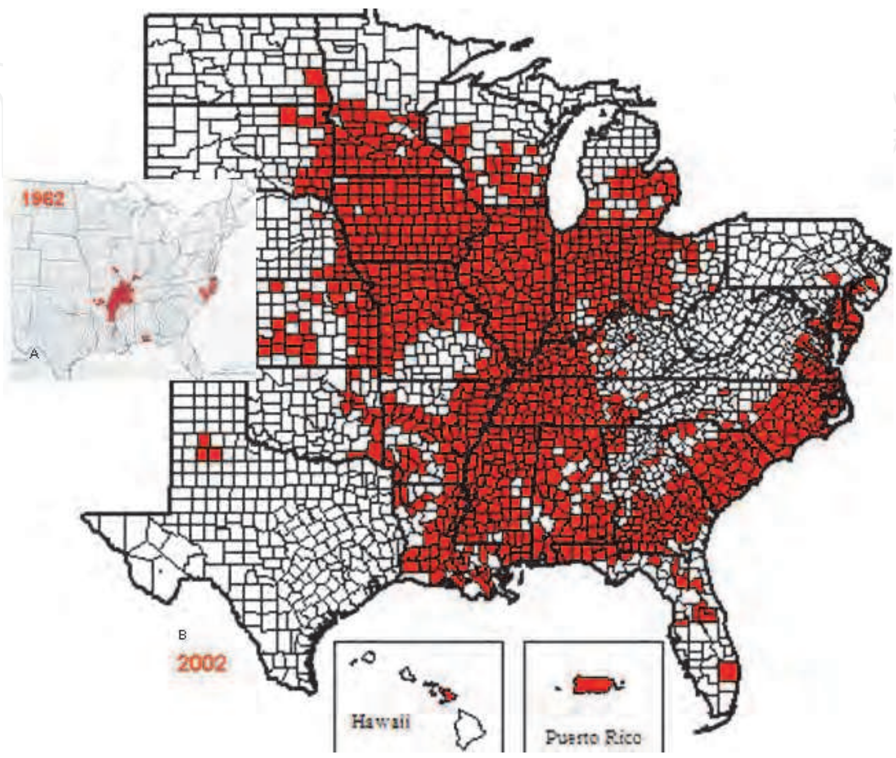

Fig. 6. Distribution map of *Heterodera glycines* in USA: A: distribution in 1962; B: distribution in 2002.

8. Host

The soybeans are the most economic important host for *H. glycines*. It has a broad host range, up to 100 plant species world wide, some selected common hosts are listed in Table 2 (Baldwin & Mundo-Ocampo, 1991), especially some legumes including beans, vetch, clover, and pea. It also attacks many species of weeds (Riggs & Hamblen, 1966). These weeds in the fields must be taken into consideration for any management measures. Some nonhosts are also listed that may be used in rotation.

9. Race

Variability in virulence among populations of soybean cyst nematode was recognized by researchers soon after the nematode was discovered in USA. The term race has been adopted by an *ad hoc* committee of the Society of Nematologists to designate the SCN populations differ in their ability to develop on a set of differential soybean cultivars (Table 3). This scheme was developed in 1970 (Golden et al., 1970) and later was refined by (Riggs & Schmitt 1988). The scheme defines 16 possible races. In 2002, HG type was introduced to more adequately define the diversity in virulence phenotypes (Niblack et al., 2002).

Host species	Non-host
Soybeans	Alfalfa
Green beans	Barley
Snap beans	Canola
Dry beans	Red clover
Red beans	White clover
Lima beans	Ladino clover
Mung beans	Oats
Bush beans	Rye
Adzuki beans	Sorghum
Graden peas	Wheat
Cowpeas	
Corn	
Vetch (common, hairy)	
Lespedeza (common, Korean, round bush, sericea)	
Birdsfoot-trefoil	
Sweetclover	
White lupines	

Table 2. A list of hosts and nonhosts for *Heterodera glycines*.

RACE	Pickett	Peking	PI 88788	PI 90763
1	-	-	+	-
2	+	+	+	-
3	-	-	-	-
4	+	+	+	+
5	+	-	+	-
6	+	-	-	-
7	-	-	+	+
8	-	-	-	+
9	+	+	-	-
10	+	-	-	+
11	-	+	+	-
12	-	+	-	+
13	-	+	-	-
14	+	+	-	+
15	+	-	+	+
16	-	+	+	+

A "+" rating is given if the number of females produced by a soybean cyst nematode population on the soybean cultivar is equal to or greater than 10% of the number produced on the susceptible cultivar Lee. If it is less than 10%, a "-" rating is given.

Table 3. Race classification scheme for *Heterodera glycines*.

Races 1, 2, 3, 4 were described in 1970 (Golden et al., 1970), race 5 in 1979 from Japan (Inagaki, 1979), and race 7 from China (Chen et al., 1988). Today a total 14 races have been reported (Table 4). Only races 12 and 16 have not been reported. USA has the most races. Race 3 is the most common race in the world.

With relative short history of growing soybean (100-200 years), the fact that USA has the highest number of races for the nematode indicates that the races were probably the results of its widespread planting of the resistant cultivars. Argentina (Doucet et al., 2008) and Brazil (Dias et al., 1998) both have a very short history of growing soybean (less than 50 years) have 6, and 9 races respectively. Very likely these races have been imported through seed stocks and on used machinery from several sources in USA. In comparison, the oriental countries, Korea (Kim et al., 1998), Japan (Ichinohe, 1988) and China (Liu et al., 1998) where soybean has been growing for several thousands years, have relative fewer races, because the modern practice of resistant cultivar selection and development is relatively new. Races 8, 11, 13, and 15 have only been found in USA. Lack of race 4 in both Japan and Korean was a surprise.

Country	Races	Total Races	Dominate Race
Argentina	1,3,5,6,9,14	6	3
Brazil	1,2,3,4,5,6,9,10,14	9	3
Canada	1,2,3,5,6	5	3
China	1,2,3,4,5,6,7,14	8	3 Northern, 4 Southern
Japan	1,3,5	3	3
Korea	1,3,5,6	4	3
USA	1,2,3,4,5,6,7,8,9,10,11,13,14,15	14	3

Table 4. Distribution of races of *Heterodera glycines* in the world

In USA, the most prevalent race for the northern states is the race 3, while the race 6 is the most common race. That difference of variability was noted along the 35 ^{0} N latitude line. Niblack and Riggs (2004) postulated that is likely caused by the history of using different resistant cultivars.

In central China lies between the Yellow and Yangtze rivers which include the province of Shandong, Anhui, Jiangsu, and Henan, and Shanxi, where the soybean may have originated, where the soybeans are planted in the summer and harvested in the fall (short growing season), the most common race is race 4 (Lu et al., 2006), race 3 has not been reported in that region. In northern China includes the province of Heilongjiang, Jilin, Liaoning, and Inner Mongolia where the soybeans are sown in spring and harvested in fall (long growing season) the prevalent race is race 3 and race 4 has only been reported in one case (Dong et al. 2008) (Fig. 7). China is only country that has these 2 races geologically separated. In the

Fig. 7. The race 3 prevalent region and the race 4 prevalent region of *Heterodera glycines* in China

race classification scheme, the race 3 and the race 4 are opposite to each other, all the 4 cultivars are resistant to the race 3 but susceptible to the race 4. The cultivars have their resistant genes rooted in the cultivars collected from the northern region of China where the race 3 is the prevalent. It is likely that these 2 races are the 2 original races and are native to these 2 regions respectively in China, with race 4 is the ancestral to the race 3, since the cradle of the ancient Chinese civilization happened to be in the race 4 region, and ancient Chinese civilization was an agricultural civilization. The north region (race 3 region) was not an ancient agricultural area, rather a nomadic region.

10. Management

10.1 Resistant cultivar

A search for sources of SCN resistance led to the evaluation of large number of the plant introduction (PI) from among exotic accession in the USDA soybean germplasm collection. Five accessions were selected as parents. The first SCN resistant cultivar "Picket" with Peking as the source for resistance, was breed and released in 1966 in USA (Brim & Ross, 1966), hundreds have been developed for all the Maturity Groups. In 1970, field populations were found readily reproduce on Picket, and Peking. That finding led to the search for another source of resistance. Chosen from USDA soybean germplasm collection, PI 88788 was used for the development of "Bedford" (Hartwig & Epps, 1978). With the widespread deployment of the SCN resistant cultivars, new races emerged. This has been the story for SCN resistant cultivar development in USA. Since the 80s, the soybean seed breeding has been transferred from public institutes to private company. At much later stage, some accessions of the Chinese soybean germplasm collection were identified have resistance. At present, Roundup ready cultivars developed by Monsanto are the most widely used cultivars in USA, and elsewhere.

Relying on few resistant cultivars alone for SCN control had been proven misguided, as the high number and shifting of the races in USA indicated (Young, 1992).

In North America, the basic management tactics of planting resistant cultivars at different fashions, and rotating with non-hosts will continue to be the main methods to manage the SCN problem, even though the tactics face great challenges of the shitting of the nematode races, and of the uneconomical of the non-hosts (Niblack & Chen, 2004).

10.2 Using mutiline cultivars for SCN management

Probably, it is hard to argue against the fact that monoculture farming has been one of causes for disease and pest epidemics, the best example is without doubt the Irish Potato Famine caused by the potato late blight disease (*Phytophthora infestans*). Recent cases of other invasive alien species such as the Dutch Elm disease also remind us that biodiversity is very important in fighting pests and diseases. The crop biodiversity used to be a norm practice before the modern agriculture (few cultivars, and a few pesticides), each farmer had to grow different kinds of crops for all household needs (grains, vegetable, and others). The usefulness of mixture of multiline cultivars and cultivars mixtures for disease control has been well documented (Mundt, 2002, Wolfe, 1985). The recent successful cases of using multiline cultivars or cultivar mixtures for controlling diseases, such as potato late blight on potato (Garrett & Mundt, 1999), on barley (Wolfe et al. 1981), on rice in China (Zhu et al., 2000) demonstrated that the practical difficulties associated with the mixtures have been overestimated. This concept has not been carefully tested for SCN control. In the few tested cases, the mixtures were not superior to the resistant cultivars in terms of their yield increasing

(Young & Hartwig, 1988). More studies are recommended. As Mundt (2002) demonstrated that for biodiversity to be functional, there must be an appropriate match between the resistant genes in a mixture and the virulence genes present in the target pathogens or parasites.

10.3 Cover crop

Cover crops are commonly used to prevent soil erosion. These crops are usually planted in rotation with primary crops. When the cover crops are incorporated into the soil at the certain stage of the growing season, this practice is being referred as green manure. A major benefit obtained from green manures is the addition of organic matter to the soil, which increases the food supply for macro, and micro organisms in the soil resulting increased biodiversity in soil. There is a lot of information on the benefit effects of soil biodiversity on disease control (Brussaard et al., 2007).

This agriculture practice with certain crops which contain nematicidal compounds is especially interesting. Marigold, especially French marigold (*Tagetes patula*) has been shown reduced the populations in soil of several root-knot nematodes, and root lesion nematodes (Motsinger et al., Ploeg, 2000, Pudasaini, 2007). Castor beans, sesame, Sudan grass, sorghum, and Crucifers have all shown are toxic against plant parasitic nematodes. Among them, plants from Brassica have received considerable attention for their possibility in controlling plant parasitic nematodes by incorporating them into soil (Mojtahedi et al., 1993, Potter et al., 1998). The principle reason is that glucosinolates which exist in these plants convert upon decomposition to isothiocyanates, a group of chemicals proven to have a wide spectrum of biological activities, including nematicidal activity (Brown & Morra 1997), a few these chemicals are volatile, the practice has been referred "Biofumigation". Among these converted isothiocynates, allyl isothiocyanate (AITC) has been proven as being the most toxic against *H. glycines* (Lazzeri et al. 1993). AITC is the decomposition product of allyl glucosinolate (generally called sinigrin), which exists in plants of *Armoracia lapathifolia, Brassica carinata, B. juncea, B. napus, B. oleracea*, and *Peltaria alliacea* (Brown & Morra, 1997). Among them, mustards have been cited most promising, especially the oriental mustard (Brassica juncea) which contains highest concentration of Ally isothiocynate (AITC) in plant (Tsao et al., 2000). AITC toxicity was found highly selective, was highly toxic against J2 of *H. glycines*, but less toxic on

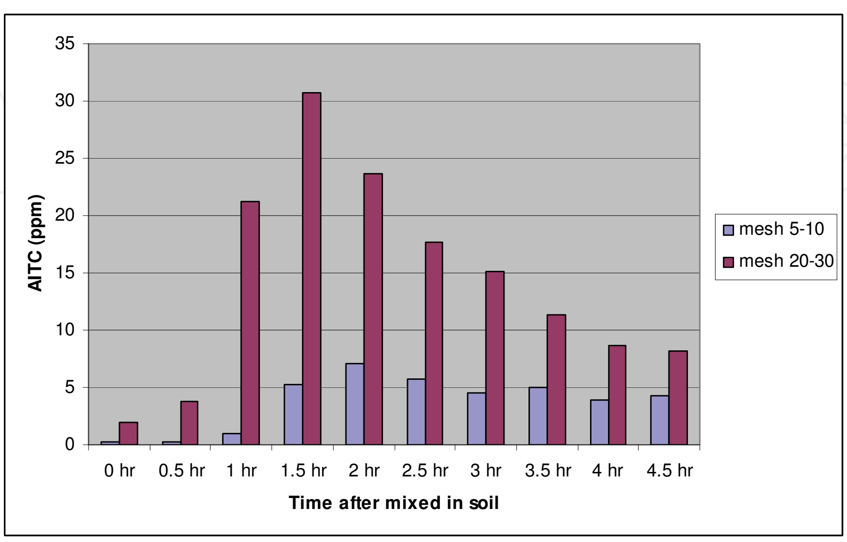

Fig. 8. Effect of particle size of mustard materials on AITC releasing in soil

free-living nematodes, AITC also inhibited the egg hatching of the nematode (Yu et al., 2005). Some materials from this oriental cultivar have been demonstrated effective in reducing population of *Pratylecnhus penetrans* in soil (Yu et al., 2007 a, 2007b).

Recently using mustard such as oil radish, or other mustard related crops as a cover crop for controlling *H. glycines* have tested, but the results have not been conclusive. The potential factors that caused the inconsistency includes: 1) targeted nematode species; 2) mustard varieties; and 3) environmental factors. In another study we found that the particle size had dramatic effect on releasing the AITC in to the soil (Fig. 8). It is likely that with a mustard variety of high AITC concentration, and plant tissue macerated to very fine particles, mustard crops as cover crops for the SCN control can be an effective method.

11. Concluding remarks

The soybean cyst nematode is more likely going to spread to new soybean growing areas around the world as the climate change intensifies, and as the world becomes more integrated. The soybean seeds are more than ever developed and marketed by a few international companies, the soybean farming practices in the world will become more and more uniform, less diverse unfortunatly. New races could emerge. This creates greater challenges for managing the SCN.

The whole genome sequencing project of *H. glycines* has been completed by Monsanto Company and Divergence. The sequencing information although has been submitted to Genbank, it remains inaccessible to the public. DOE Join Genomic Institute led by Kris Lambert and Matthew E. Hudson (Univ. of Illinois at Urbana-Champaign) is in the process of sequencing the pest as well, in a hope that it will lead us to learn more about the races, and to find new ways for the controlling of the pest.

There are a few soybean germplasm collections in the world with the USDA collection being the largest, the holding information is accessible to the public. The Chinese soybean germplasm collection holds 6644 accessions of *Glycine soja*, a potential rich pool of source of resistance. Collaborations between the collections such as sharing information and germ lines are essential. The management of SCN must not rely on a few resistant cultivars. An integrated approach involving several cultivars, rotating with nonhosts, and cultivation practices that encourage biodiversity in the soil must be the future.

12. Acknowledgment

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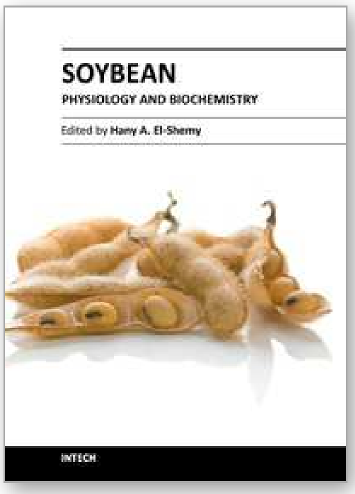

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Worldwide, soybean seed proteins represent a major source of amino acids for human and animal nutrition. Soybean seeds are an important and economical source of protein in the diet of many developed and developing countries. Soy is a complete protein and soyfoods are rich in vitamins and minerals.Soybean protein provides all the essential amino acids in the amounts needed for human health. Recent research suggests that soy may also lower risk of prostate, colon and breast cancers as well as osteoporosis and other bone health problems and alleviate hot flashes associated with menopause. This volume is expected to be useful for student, researchers and public who are interested in soybean.

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