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Nutritional Requirements of Soybean Cyst Nematodes

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1. Introduction

Soybeans [*Glycine max*] are the second largest cash crop in US Agriculture, but the soybean yield is compromised by infections from *Heterodera glycines*, also known as Soybean Cyst Nematodes [SCN]. SCN are the most devastating pathogen or plant disease soybean farmers confront. This obligate pathogen requires nutrients from the plant to complete its life cycle. To date, SCN nutritional requirements are not clearly defined. Growth media supporting SCN still contain soy products. Understanding the SCN nutritional requirements and how host plants meet those requirements should lead to the control of SCN infestations. The nutritional requirements of SCN are reviewed in this chapter and those requirements are compared to those of other nematodes. Carbohydrates, vitamins, amino acids, lipids, and other nutritional requirements are discussed.

The survival of parasitic nematodes requires adequate nutrition. These essential nutrients are at least partially supplied by the host. But, availability of nutrients may not alone be sufficient for survival and reproduction. The parasite must also be able to establish a feeding site. Both the establishment of the feeding site and the presence of adequate nutrients for the soybean cyst nematode [SCN] are discussed below.

1.1. Feeding site establishment

Nematodes have differing mouth part structures which are adapted to their food source [1]. In the case of plant-parasitic nematodes, a stylet [analogous to a hypodermic needle], is used to puncture plant cells and a pump mechanism located in the nematode esophagus allows for exchange of fluids between the nematode and plant [1]. Most studies of the economically important root-knot and cyst-forming plant-parasitic nematodes have focused on what fluids are secreted by the nematode and how this facilitates establishment of a feeding site [2-4].

Specific information on the essential nutrients provided by the plant is lacking. In this chapter we focus on what is known about nutrient requirements for soybean cyst nematode, SCN.

The SCN is an obligate parasite requiring a host plant to complete its life cycle (see Figure 1). The cysts are found in the soil and contain eggs and first stage juveniles. The second stage juvenile hatches from the egg and penetrates plant roots. If the roots are a plant that is a host for SCN, the third and fourth stage juveniles molt into an enlarged shape called a sausage once a feeding site is successfully established where the primary goal is removing nutrients from the plant for use by the nematode. After enough nutrients have been obtained by the nematodes, those destined to become males molt into a worm-shape again and migrate out of the roots in search of a female. As the females mature, their size increases breaking root epidermal cells and the nematode is exposed to the soil where she emits pheromones to attract the males already in the soil. Once fertilization of the eggs has occurred, the female dies and her hardened body becomes the cyst which protects the eggs from environmental extremes and organisms which can kill the eggs. Some eggs are extruded into the soil in a gelatinous matrix and these eggs are thought to hatch once conditions favor hatch. The eggs within the cyst go through diapause and can survive within the cyst for more than a dozen years under the right conditions. Juveniles which enter nonhost plant roots may molt into a third stage juvenile but a successful feeding site will not be established and the plant will recognize the nematode as an invader and form necrotic cells surrounding the nematode effectively killing the nematode. Alternatively, some plants are slower to recognize the nematode as an invader and a molt to the third stage may occur but no further development of the nematode will occur. Once the nematode reaches the sausage stage, it lacks the muscles to leave the root and it dies.

As an important crop in the United States [5], there are over 120 soybean lines which have some level of resistance to SCN [6]. Commercial soybean varieties primarily contain one or more different sources of resistance but 95% of all resistance is found from one source, PI 88788. Peking [PI 548402] and Hartwig [PI 437654] are also found in a few commercial varieties. Genetics of resistance is complex with multiple genes involved and interaction of minor genes or nongenetic sources complicates understanding of the process. In a resistant reaction, cytological changes occur and these have been documented [7-19]. Initial reaction to the nematode during the formation of the syncytium in both susceptible and certain resistant lines is identical for the first 4 days after infection [7, 9, 11]. Resistant reactions can be seen about day 4-5 [7, 9-11].

Cyst nematode juveniles hatch from eggs within the cyst or in the soil and enter plant roots typically in the zone of root elongation. They migrate to the pericycle and establish a feeding site [20]. Cellulases break polysaccharide chains and associated proteins in the plant cell walls. Other enzymes have been shown to be secreted by the nematodes as they move through plant tissue [21]. Rapid response by the plant to the nematode inhibits formation of a successful feeding site. A successful feeding site initiation results when the plant fails to respond or responds slowly to the presence of the nematode. One of the ways plant-parasitic nematodes protect themselves from plant responses to the nematodes is through secretion of peroxiredoxin, glutathione peroxidase, and secreted lipid binding proteins within the surface coat of the nematode [22]. Although considerable knowledge is now available on the morphological

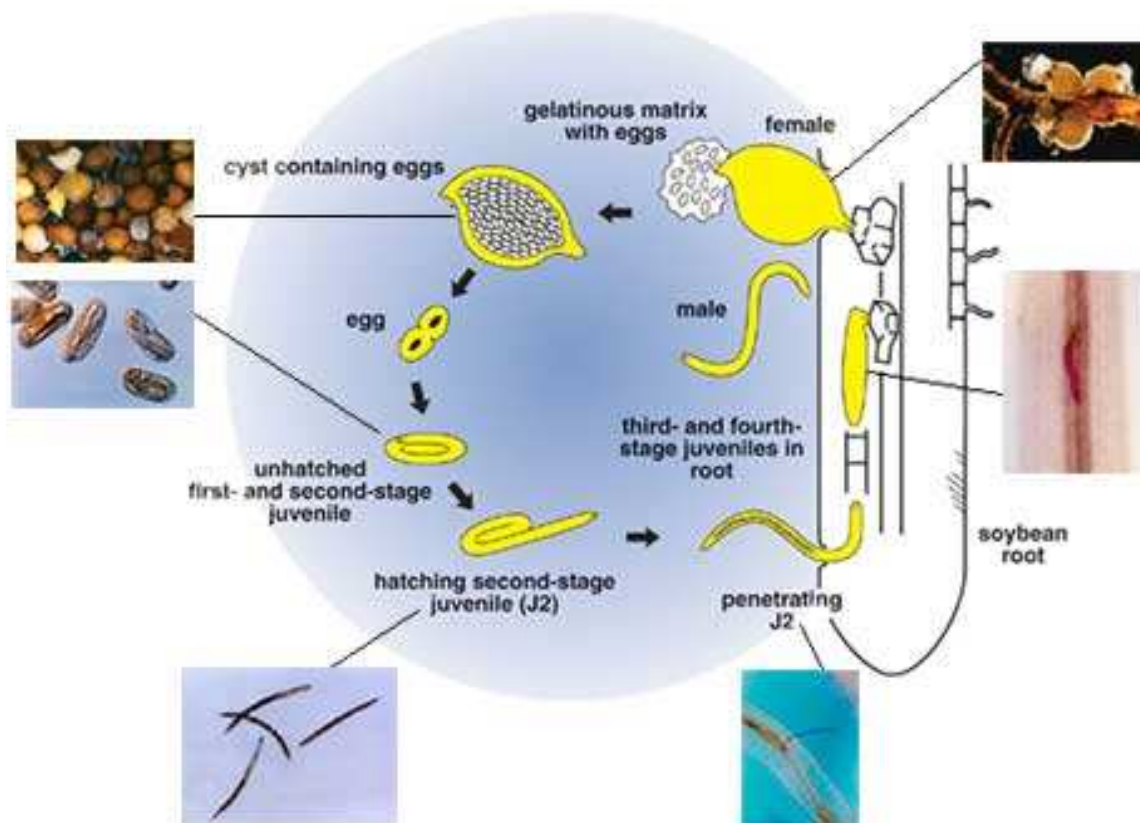


Figure 1. The life cycle of the soybean cyst nematode (SCN) is shown. Soil contains cysts with eggs as well as first stage juveniles. Second stage juveniles hatch from eggs and can then penetrate plant roots. The third and fourth stage juveniles feed off the plant. Males migrate out of the roots in search of a female. Maturing females rupture the root, releasing pheromones to attract males from soil. Females die after egg fertilization and her body becomes the cyst. This figure was obtained with permission from www.extension.umn.edu.

changes in the plant cells due to the presence of the nematode feeding site and molecular studies have advanced our understanding of the interactions on a molecular level, the details of host specificity are unknown [23].

Information is available on the changes that occur within soybean plants when a compatible interaction between SCN and the plant occur. Information is also present on incompatible reactions when plant resistance inhibits SCN reproduction through either a hypersensitive response or formation of small syncytia which limit SCN reproduction. Infection of plant-parasitic nematodes is thought to alter plant products from the shikimic pathway. Infection by SCN increases the concentration of glucose, K, Ca and Mg in the roots but information is not available on whether these increases are products SCN then extracts from plant cells or whether these are responses by the plant to the presence of the nematode.

1.2. Nutritional requirements

Heterodera glycines is considered to have a wide host range. Riggs and Hamblen tested 1152 entries from the Leguminosae family and found that 399 of these entries from 23 genera were susceptible. Poor hosts included 270 entries in 12 other genera [24]. Additional host studies

have been conducted by Riggs and Hamblen [25-26], Miller and Gray [27-28], Venkatesh et al, [29], and Venkatesh et al [30]. Variability in host status within a plant species potentially makes identification of necessary nutrients required for establishment of the obligate feeding site easier but to date the specifics have eluded scientists.

A summary of the plants invaded by SCN are shown in Table 1. Most hosts of SCN are legumes and are limited to three subfamilies of the Leguminosae; however, approximately 50 genera in 22 families including nonlegumes are also hosts [31-32]. Some plants allow SCN to penetrate plant roots but limit reproduction of SCN [33]. The reason for this could be nutritional, or it could be due to other barriers within the plant. To determine which of those two possibilities are controlling virulence of SCN, nutritional requirements should be investigated more fully.

Host Common Name	Host Scientific Name	Use
azuki bean	<i>Vigna angularis</i>	edible
. bean tree	<i>Laburnum sp</i>	ornamental
beans, green, dry	<i>Phaseolus vulgaris</i>	edible
beard tongue	<i>Penstemon digitalis</i>	ornamental
begger tick	<i>Desmodium ovalifolium</i>	weed
bells of Ireland	<i>Mollucella laevis</i>	ornamental
bitter cress	<i>Barbarea vulgaris</i>	spice
bladder senne	<i>Colutea arborescens</i>	shrub -ornamental
bush clover	<i>Lespeza capitata</i>	prairie plant
California burclover	<i>Medicago hispida</i>	weed
common chickweed	<i>Stellaria media</i>	weed
common lespedeza	<i>Lespedeza striata</i>	weed
coral bells	<i>Heuchera sanguinea</i>	ornamental
cranesbill	<i>Geranium maculatum</i>	weed
largeflowered beardtongue	<i>Penstemon gradiflorus</i>	wildflower
field pea tuberous vetch	<i>Lathyrus tuberosus</i>	edible/weed
fennugreek	<i>Trigonella goenum-gracum</i>	spice
foxglove	<i>Digitalis sp.</i>	weed
. geranium	<i>Pelargonium sp</i>	ornamental
gold apple	<i>Lycopersicon esulentum</i>	weed
golden chain	<i>Laburnum anagyroides</i>	ornamental

Host Common Name	Host Scientific Name	Use
grass pea vine	<i>Lathyrus sativa</i>	edible/ornamental
green pea	<i>Pisum sativum</i>	edible
hairy vetch	<i>Vicia villosavillosa</i>	forage /cover crop
hemp sesbania	<i>Sesbania exaltata</i>	weed
henbit	<i>Lamium amplexicaule</i>	weed
hog peanut	<i>Amphicarpa bracteata</i>	weed
Indian joint vetch	<i>Aeschynomene virginica</i>	weed
indigo	<i>Indigofera parodiana</i>	shrub/herbaceous/small tree
clover Kenyan clover	<i>Trifolium</i>	ornamental
Korean lespedeza	<i>Lespedeza stiuacea</i>	forage
lance leaf rattlebox	<i>Crotalaria lanceolata</i>	weed
large flowered beard tongue	<i>Penstemon grandiflorus</i>	wild flower
large leaf lupine	<i>Lupinus polyphyllus</i>	wild flower
licorice milk vetch	<i>Astragalus glaucophyllus</i>	forage
little bur clover	<i>Medicago minima</i>	weed
milk vetch	<i>Astragalus canadensis</i>	forage
milky purslane	<i>Euphorbia supine</i>	weed
mouse ear chickweed	<i>Cerastium vulgatum</i>	weed
Common mullein	<i>Verbascum thapsus</i>	weed
nasturtium	<i>Tropaelum pergrinum</i>	ornamental
old field toadflax	<i>Linaria canadensis</i>	weed
pigeon pea	<i>Cajanus cajan</i>	edible
Americana pokeweed	<i>Phytolacca</i>	weed
purple deadnettle	<i>Lamium purpureum</i>	weed
purslane	<i>Portulaca oleracea</i>	weed
rainbow pink	<i>Dianthus chinensis</i>	ornamental
river bank lupine	<i>Lupinus rivularis</i>	edible
Rusian sickle milk vetch	<i>Astragalus falcate</i>	weed
service lespedeza	<i>Lespedeza cuneata</i>	weed
shrub lespedeza	<i>Lespedeza bicolor</i>	ornamental

Host Common Name	Host Scientific Name	Use
Siberian pea tree	<i>Caragana arborescens</i>	ornamental
sicklepod	<i>Cassia tora</i>	weed
small flowered buttercress	<i>Cardamine parviflora</i>	weed
soybean	<i>Glycine max</i>	edible
Spanish broom	<i>Spartium junceum</i>	ornamental
speedwell	<i>Veronica peregrine</i>	weed
spider flower	<i>Cleome spinosa</i>	ornamental
spotted burclover	<i>Medicago arabica</i>	forage
stinking clover	<i>Cleome serrulata</i>	weed
sweet clover	<i>Melilotus taurica</i>	weed
sweet pearl lupine	<i>Lupinus mutabilis</i>	edible
tiny vetch	<i>Vicia hirsute</i>	ornamental vine
white horsehound	<i>Marrubium vulgare</i>	medicinal plant
white lupine	<i>Lupinus albus</i>	livestock feed
white pea	<i>Lathyrus ochrus</i>	wild flower
Wilcox penstemon	<i>Penstemon wilcoxi</i>	wilflower
winged pigweed	<i>Cycloloma atriplicifolia</i>	weed
yellow lupine	<i>Lupinus lateus</i>	wild flower

Table 1. Common names for plants that have been identified as good hosts for soybean cyst nematode [24-31].

In many ways, it is inappropriate to compare humans to nematodes. But, from a nutritional perspective, much more is known about human nutrition than what is known about nutritional requirements of nematodes. For humans, numerous biochemical and mineral components are essential nutrients. But, for nematodes, only a few are known. Yet, nematodes have a comparatively simple digestive system. So, it would be reasonable to predict that nutritional requirements for these organisms are more extensive than what is currently known.

It is also inappropriate to generalize nutritional needs from studies on one nematode to all the nematodes within the various trophic categories. Certainly there should be similarities, but it is clear from the literature that animal parasitic nematodes have different needs from the plant parasites. And, it may also be that those plant parasites infecting specific organisms, such as SCN might have nutritional needs that synergize with the contents of the host soybean plant.

Survival is best understood when chemically defined culture media can be shown to not only sustain life, but also to promote reproduction. Chemically defined media have been identified for the survival of some nematodes and this work has recently been reviewed [34]. The

successful media originally included all the amino acids in *Escherichia coli*, and in the amino acid ratios found in *E. coli*. Nematode growth media has been since modified to include a greater number of constituents including glucose, minerals, growth factors, nucleic acid precursors, vitamins, a sterol and heme source. However, SCN has not yet been shown to survive or reproduce on these media. Currently, the only growth media known to sustain SCN includes soy products [35].

Articles published on the nutritional requirements of a wide range of nematodes, generally do not specify SCN [1. 36-37]. While a few nutritional requirements for individual nematode species have been studied, these requirements are limited and their applicability to SCN is unknown. It is assumed that plant- and animal-parasitic nematodes may have different nutritional requirements from entomopathogenic, and microbivorous nematodes.

2. Lipids

Lipids consist of many non-water soluble components including free fatty acids, phospholipids, triglycerides, sterols, and other species. Many of these classes have been studied at least in one host-nematode relationship and are the most studied with the exception of nucleic acids due to their great structural variety and importance as food reserves. For example, Krusberg [38] reported the total lipids and fatty acids from 5 species of plant parasitic nematodes, and their common hosts. They found that the nematodes had the same fatty acids as the hosts, with the exception of the polyunsaturated fatty acids. These appeared to be synthesized by the nematodes. There was also some speculation that nematode fatty acid synthesis resembled that of bacterial pathways rather than that of higher animals. It was not clear from the study whether intestinal flora of the nematode could have been at least partially responsible for this difference, or whether the nematode itself synthesized the fatty acids. Some nematodes are clearly capable of synthesizing longer chain fatty acids from shorter chain precursors. They are also capable of desaturating the fatty acids [39].

Entomopathogenic nematodes infecting locusts consume host fat and protein [40]. A decrease in lipid reserves has been seen in starved nematodes which can be related to decreased infectivity [41]. Lipid content is also known to decrease when nematodes come out of anhydrobiosis [42]. Lipids associated with the nematode surface [cuticle] are triacylglycerols, sterols, specific phospholipids, and other glycolipids [43-45].

The most widely known class of essential nutrients for nematodes is sterol [36,46]. This nutritional requirement was first discovered by Dutky et al. [47] and thought to be potentially a means for control of plant parasitic nematodes. A recent review further confirms this nutritional sterol requirement for the nematode *C. elegans* [48]. Nematode parasites of animals also require sterol for larval development [49]. The biochemical mechanism which converts sitosterol to cholesterol appears to be lacking in nematodes [50]. Nematodes are capable of modifying sterols obtained from their diet [46] but degradation of sterols to CO₂ by nematodes is not clear [51]. More than 63 sterols have been identified from free-living and plant-parasitic nematodes. Characteristics of sterols which can be used by nematodes include those which

have a hydroxyl group at C-3, a trans-A/B ring system and an intact nonhydroxylated side chain but lack methyl groups at C-4 [52]. Plant sterols are different than animal sterols with plants being unique in methyl, ethyl or related alkyl groups at the C-24 position of the sterol side chain [52]. There are also differences between plant sterols and plant-parasitic nematode sterols. These findings suggest that nematodes ingest plant sterols and remove the C-24 side chain. In addition, the nematode saturates the double bonds in the four-membered ring system to produce stannols [52]. Steroid hormones are important in development processes and in transition to different life stages [53]. Most likely genetic and biochemical methods will be needed to determine the function of hormones found in nematodes [54]. Novel genes involved in the production of 17 β -hydroxysteroid dehydrogenase in the soybean cyst nematode have been reported [55].

Sterols were first reported in soy oil by Kraybill et al. [56]. Formononetin is an o-methyl-isoflavone mainly produced in legumes, including soybean plants [57]. It helps stimulate the production of steroids in mammals, and possibly also in nematodes. Research in this area by the USDA was reviewed by Chitwood [58].

3. Amino acids and proteins

There are no clearly defined requirements for proteins, amino acids, or peptides for SCN. However, it is unlikely that nematodes synthesize all the amino acids. For humans, there are 9 essential amino acids [phenylalanine, valine, threonine, tryptophan, isoleucine, methionine, leucine, lysine, and histidine]. Some others are required under special circumstances [arginine, cysteine, glutamine, proline, serine, tyrosine, and asparagine]. Cysteine, tyrosine, and arginine are required during rapid growth, such as in infancy. And, arginine, cysteine, glycine, glutamine, histidine, proline, serine and tyrosine are required by some individuals because these amino acids are not adequately synthesized by these individuals. These are essential components for the synthesis of many essential enzymes and structural proteins ; it is anticipated there are similar needs in the nematode diet.

Protein consumed by parasitic nematodes can severely damage the host. Juveniles have high protein requirements and consuming the host protein can severely weaken the plant [46].

There have been efforts to identify the essential amino acids of nematodes [59-61], but so far common requirements have not been identified. However, protein synthesis in cotton roots is modified when the root-knot nematode [RKN] infects susceptible plants. These plant-parasitic nematodes influence the distribution of amino acids in cotton root galls [61]. Also, there is one genetic modification of the cotton plant which makes them less susceptible to infection by the RKN. This modification is responsible for the synthesis of a 14 kDa protein [60].

For the snail parasitic nematode, *Rhabditis maupasi*, five essential amino acids have been identified. These include lysine, methionine, phenylalanine, tryptophane, and valine [62]. In the entomophilic locust parasite, *M. migrescens*, essential nutrients include protein nitrogen [63]. Essential amino acids have also been identified for the nematode *C. briggsae* [64].

4. Vitamins

There are 13 essential vitamins required by humans. These include Vitamin A [Retinol] Vitamin B₁ [Thiamine] Vitamin C [Ascorbic acid] Vitamin D [Calciferol] Vitamin B₂ [Riboflavin] Vitamin E [Tocopherol] Vitamin B₁₂ [Cobalamins] Vitamin K₁ [Phylloquinone] Vitamin B₅ [Pantothenic acid] Vitamin B₇ [Biotin] Vitamin B₆ [Pyridoxine] Vitamin B₃ [Niacin] Vitamin B₉ [Folic acid]. Of these, vitamin E is known to be a nutritional requirement for the gastrointestinal parasite, *Heligmosomoides bakeri* [65], and several of the B vitamins are known to be essential nutrients of *C. elegans* [66-68].

For SCN, DNA sequences responsible for the biosynthesis of enzymes that can produce some of the B vitamins *de novo* have been discovered [69]. Therefore, SCN may not need the same B vitamins as *H. bakeri*, for example. And, it is likely that there are other differences in vitamin and supplement requirements across all nematodes.

5. Minerals

Considerable research on mineral requirements for nematodes has been reported in mammalian parasites. For example, the gastrointestinal nematode, *H. bakeri*, requires boron [70], zinc [71], and selenium [65] for survival. And, other nematodes have similar mineral requirements [72-74]. For example, magnesium, sodium, potassium, manganese, calcium and copper are required nutrients of *C. elegans* [5]. However, SCN mineral requirements remain unclear.

Whether minerals, influence nematode survival may not help in their control if necessary minerals are readily available in soil, and essential to the host organisms. But, elements not essential to survival of the host could be controlled in soils to help control SCN survival.

6. Carbohydrates

Nematodes require carbohydrates for energy, usually in the form of glycogen. One study showed that several different carbohydrates were sufficient to provide a carbon, or energy source for *C. elegans*, and that glucose was more effective than fructose or sucrose [76]. For *C. elegans*, glucose along with cytochrome c and β -sitosterol were sufficient to sustain a healthy population.

One of the most striking features of soybean chemistry is the abundance of pinitol [77-79]. Pinitol is a carbohydrate with unusual nutritional properties [77]. Figure 2 shows a total ion chromatogram of a derivatized extract of soybean roots. It is unusual for a plant to have so much pinitol. The levels shown in this study indicate pinitol is present at a concentration of 26 mg/g (dry weight) compared to peanuts with only 4.7 mg/g or clover with 14 mg/g [79]. However, there is no evidence that pinitol, or any of the related inositols are needed for SCN survival [79].

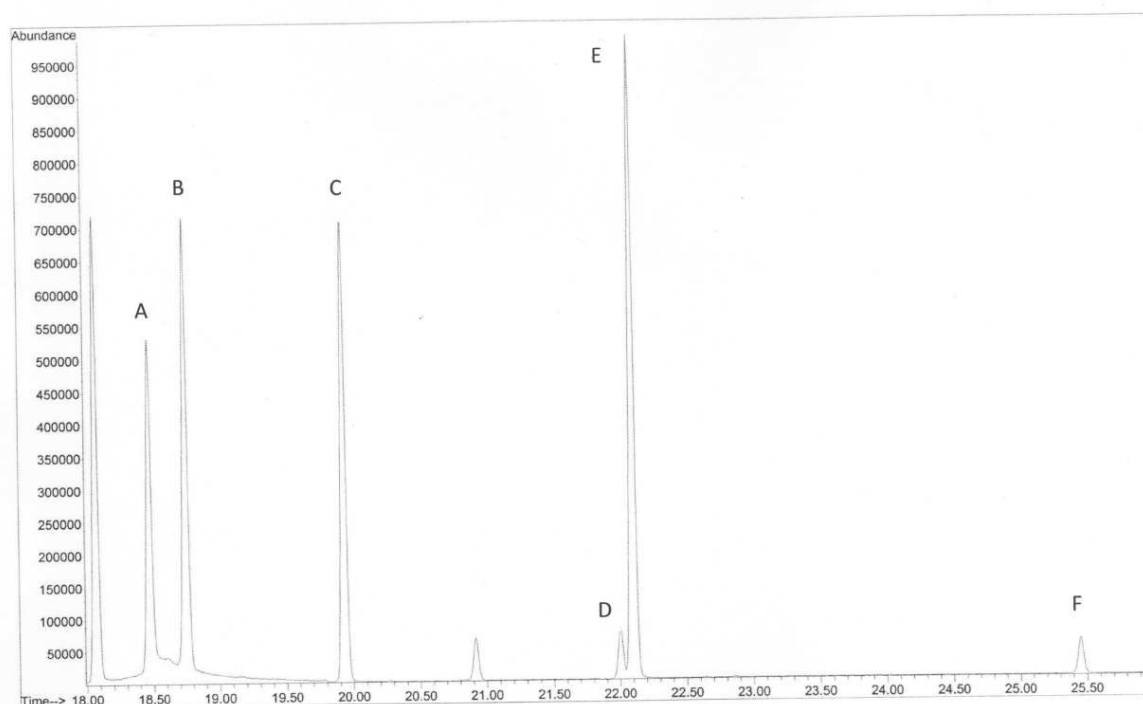


Figure 2. A total ion chromatogram of derivatized soybean root extract is shown. A = D-(-)-Fructose, B = D-Pinitol, C = D-(+)-Glucose, D = D-*chiro*-inositol, E = β -D-(+)-Glucose, F = *Myo*-inositol. Reproduced with permission from [79].

7. Other nutrients or feeding requirements

The nematode *Rhabditis maupasi* requires hemin or another iron porphyrin for survival [62]. Similarly, *C. elegans* also requires a heme source for survival [34]. It is likely that many other nematodes require heme, or a closely related hemin. There is also good evidence that SCN requires a heme source [80].

8. Discussion

In comparison to our knowledge of human nutrition, our understanding of nutritional requirements of SCN is in its infancy. Limited information is available for members of the Nematoda Phylum, but such a small amount of information is available that extrapolation across trophic groups and even within genera may be misleading. Finding a successful artificial diet would be a reasonable first step in defining the nutritional needs of SCN. But, this data needs to be coupled with a good understanding of feeding site establishment and plant responses to SCN infections.

Studying biochemical pathways would be a valuable approach, and could also help identify pathways that could be blocked to help minimize SCN survival. Our laboratory began by examining the chemistry of the plant to identify unique nutrients necessary for SCN survival, but that approach was not immediately successful. Another approach is to continue to use DNA mapping to better understand potential plant and parasite pathways. While this approach is less direct, it is currently a very active area of investigation, and can reveal more information than simply nutritional requirements.

Details of the SCN host-parasite responses during infection and feeding site establishment have been more extensively investigated than nutritional requirements. Relationships between the available nutrients from host plants compared to non-hosts could provide valuable clues on these requirements. And, once an adequate media for SCN survival has been well defined, methods to control this pest should follow.

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