# INTRODUCTION TO NEMATODES

E.C. McGawley[1], C. Overstreet[2], M.J. Pontif[3] and A.M. Skantar[4]

[(1-3)]Nematologists, LSU AgCenter, Baton Rouge, LA 70803 and [4]Research Molecular Biologist, USDA-ARS Nematology Laboratory, Beltsville, MD 20705

(July, 2011)

This document contains slide numbers, comments on content where necessary, and explanations for abbreviations used on slides in this presentation, "Introduction to Nematodes." Credits for materials that are not acknowledged on slide number 122 are included herein. All elements associated with this presentation are for use for non-profit, educational purposes in the fields of plant nematology, plant pathology and related plant protection and helminthology disciplines. The primary vehicles for the dissemination of this presentation are anticipated to be The Society of Nematologists and The Organization of Nematologists of Tropical America. Users and viewers are urged to visit the websites of these two organizations (http://www.nematologists.org and http://ontaweb.org) to learn more about their contributions to science and agriculture and to become familiar with the philanthropic activities of the N.A. Cobb and ONTA Foundations.

Materials in this presentation are focused primarily on nematodes that are parasitic on agriculturally important plants. Observers and narrators of this presentation should be mindful of and convey to audiences the great diversity and immense importance of other members of this unique assemblage of animals, certainly given unjustifiably minor emphasis herein, grouped into Nematoda. Feedback from users of this presentation is encouraged via the email contacts at the end of this document. Contributions of photographs or formatted data that improves existing sections or adds new sections on nematodes other than those that are plant parasites are most welcome. This presentation should NOT be considered a FINISHED product. Hopefully, with contributions from individuals interested in nematodes, it will evolve into an ongoing project that reflects the biological, ecological, and scientific knowledge accumulated about nematodes and their roles in nature.

1.) **Introduction**. The purpose of this introductory slide is to convey the fact that nematodes inhabit almost every known ecological niche on earth. Nematodes are among, if not the, most abundant multicellular animals on earth. The first invertebrates appeared ca. 600 million years ago; with fossilized specimens from amber in Lebanon indicating the first nematodes (insect parasitic mermithid nematodes) from 135-120 million years ago (Poinar, G.O. et al. 1994. Fundam. Appl. Nematol., 17(5) 475-477).

2.) **The Six Kingdoms**. Data here illustrate the abundance and groupings of living things on earth from the "Tree of Life web project" (http://tolweb.org/tree/). Note

that animals account for one million of the 1.5 million living things described to date.

3.) **Worms**. Establishing the relative positions of worms and worm-like creatures within the kingdom Animalia. Animals display either radial or bilateral symmetry. Bilaterally symmetrical organisms are either deuterostomes or protostomes and the first "Build-in" explains these terms and provides the embryology necessary for an understanding of the formation of the mesoderm tissue present in a triploblastic organism. Both worms and worm-like animals are protostomes and divide into two "superphyla:" those that contain "animals with external cilia and/or filter combs" (Lophotrochozoa) and those that "molt and have cuticles" (Ecdysozoa). Details are at http://www.wormbook.org/toc_nematodeevolecol.html in the second chapter by Paul DeLey. There are nine phyla (Platyhelminthes, Bryozoa, Sipuncula, Mollusca, Nemertea, Entoprocta, Annelida, Phoronida and Brachiopoda) of Lophotrochozoa and eight phyla (Arthropoda, Onychophora, Tardigrada, Nematomorpha, Kinorhyncha, Loricifera, Priapulida and Nematoda) of Ecdysozoa. From this point, Build-in 2 shows the (cestode) tapeworm, *Taenia pisiformis*, it's anterior end (scolex), attached to the intestine of a rabbit (www.thiagoodview.com).

4.) **Phylum Nematoda**. The six Build-ins introduce characteristics of animals in the phylum Nematoda. The terms coelomate, pseudocoelomate and acoelomate are explained in Build-in 1. **Note:** Some experts contend the Pseudocoelomata is an artificial paraphyletic group. However, the pseudocoelom is still a character of nematodes and is presented here only for an overview and is not meant to endorse the phylogenetic validity of the group.

5.) **Animal Parasites**. Top right: river blindness caused by *Onchocerca volvulus.* This nematode is vectored by the blackfly, *Simulium damnosum*; Center right: *Toxocara canis*, the dog roundworm; Bottom right*: Toxocara cati* from common house cat (modified from petcaregt.com/cat-worm.html); Bottom left: heartworm, *Dirofilaria immitis;* Top left: the hookworm, *Ancyclostoma duodenale*; Build-ins: 1.) the nematode, *Loa loa,* being removed from the eye; 2.) the Guinea worm, *Dracunculus medinensis,* being removed from a foot*;* 3.) the symbol of medicine, the caduceus, said by some to represent the Guinea worm and a tool used for its removal from a human (note: outside of America, many professional and patient centered organizations use the staff of Asclepius that has a single serpent encircling the staff as their symbol); 4.) swollen leg of an individual infected with the elephantaisis nematode, *Wuchereria bancrofti*; 5.) biblical references to nematode infections by the Guinea worm, *Dracunculus medinensis* and human cutaneous larval migrans caused by the hookworms *Ancylostoma duodenale* and *Necator americanus*; 6.) colorized ascarid poultry worm.

6.) **Free-Living Species**. Top right: electron micrograph of anterior of *Acrobeles* sp.; Center right: *Caenorhabditis elegans*; Bottom right: *Mononchus* sp. feeds on another nematode; Bottom center: video; Left: *Dorylaimus* sp.; Build-ins: 1 A.) *Thoracostoma* sp.; B.) *Acromoldavicus mojavicus*; C.) *Enoploides* sp.; D.)

*Pontonema* cf. *parpapilliferum*; E.) *Ceramonema* sp.; F.) *Latronema* sp.; G.) *Actinca irmae*; 2.) *Mononchus* sp. feeds on another nematode.

7.) **Marine Inhabitants (including parasites of marine fauna)**. Top right: *Rhabditis* sp.; Bottom right: *Eustrongyloides* sp. from the Northern Snakehead fish, *Channa argus*; Bottom center: *Trissonchulus* sp.; Bottom left: drawing of a male *Glochinema bathyperuvensis* (dpc.uba.uva.nl); Middle left: *Onyx* sp.; Top left: unknown marine species (www.arcodiv.org); Build-in: (top) *Phocanema* sp. lying on fish filet; (bottom): anterior of *Camallanus cotti*.

8.) **Plant Parasites**. Top left: (red esophagus) colorized illustration from B.Y. Endo; Build-ins 1-6: major characteristics of plant parasitic nematodes; 7.) diagram illustrating the relative sizes of the most common genera of plant parasitic nematodes (modified from Plant Pathology by G.N. Agrios, 5th Edition, 2005, Elsevier Academic Press); 8.) preliminary introduction to nematode anatomy. **Note:** Some experts contend that 90% of nematodes are marine, making plant and animal parasites by comparison a tiny minority. Additionally, students should be aware that most plant parasitic nematodes are not agricultural pests but simply a part of natural ecosystems.

9.) **Etiological Agents of Plant Diseases**. For each pathogen group, the chronological order of discovery, the first disease and discoverer are presented. (Model: available from Mactode Publications). Cell diagram from Plant Pathology by G.N. Agrios, 5th Edition, 2005, Elsevier Academic Press.

10.) **The Tool of the Trade**. C= cone, S= shaft and K= knob. DEGO= dorsal esophageal gland orifice, EL= esophageal lumen, A= ampulla. Build-out: stylet overlays. Build-ins: 1.) functions of the stylet; 2.) note of caution; 3.) Illustration of stylets of Tylenchs, Trichodorids and Dorylaims (Trichodorid is *Paratrichodorus hispanus* from F. Roca and M. Arias [Nematol. Medit. 14:181-185]); 4.) video: *Bursaphelenchus xylophilus*, the pinewood nematode, feeding on mycelium of the fungus *Gliocladium virens* (real-time).

11.) **Life Cycle**. Illustrated are eggs (single, in egg masses and in a cyst, undifferentiated and fully differentiated); hatching juveniles (ectoparasitic and endoparasitic); maturing individuals; and adults (females and tail sections of males showing spicules). Build-ins: 1.) root-knot juvenile hatching from egg (400X in real time); 2.) reniform juvenile hatching from egg (1000X in real time); 3.) nematode life cycle durations. Note: The second stage juvenile (J2) is the infective stage for most plant parasitic nematodes. However, this is not the case with all plant parasites such as *Rotylenchulus reniformis*, where the preadult female is the infective stage.

12-14.) **A Brief History of Plant Nematology**. Notable background elements are Ebers Papyrus and a pinworm (*Enterobius vermicularis*) specimen from picturesofparasites.com; Build-ins: 1.) symptoms of nematode infestation on infested grain heads; seed (dark); second stage juveniles emerging from grain;

and juveniles (bottom) in anhydrobiotic state ( from M. McClure); 2.) verbatim text from Needham's writing on the subject. Slides 13 & 14 are self-explanatory.

15.) **A Phenetic Grouping of Nematodes**. Build-in: *Scutellonema brachyurum*, the original classification of nematodes was based upon the presence or absence of phasmids (shown stained in the photograph).

16.) **A Phylogenetic Grouping of Nematodes**. Characteristics of Adenophorea and Secernentea. Location of the dorsal esophageal gland orifice (DEGO) in Tylenchina and Aphelenchina. Build-ins: the phylogenetic grouping of nematodes as presented in De Ley, P. A quick tour of nematode diversity and the backbone of nematode phylogeny (January 25, 2006), *WormBook*, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.41.1, http://www.wormbook.org. Another excellent website for consultation is http://insects.tamu.edu/research/collection/hallan/Nematoda/Family/0NematodaIndex0.htm.

17.) **Identification**. Importance of the esophagus (referred to by some as the oesophagus or simply, pharynx) in the identification of nematodes; Build-ins: 1.) criconematoid esophagi; 2.) humor; 3.) parts of the esophagus; 4.) cross-section through Tylenchid nematode in the area of the median bulb showing the esophageal lumen; 5.) animation of the median bulb pump (P.M. Sforza and J.D. Eisenback).

18.) **Morphology**. The illustrations of male and female nematodes are not indicative of any particular genus. They are "hybrids" intended to provide students with knowledge of the basic external morphological features common on most plant parasites. Note: DeMan's values, employed in the identification of genera and species, are based on measurements of specific body regions of the nematode. Build-ins: 1.) additional DeMan values; 2.) cuticle and lateral field; 2.) head region (en face); 3.) types of sensilla.

19.) **Internal Body Systems**. Build-ins: 1.) types of reproduction by plant parasitic nematodes; 2 & 3.) monodelphic and didelphic ovaries; 4.) terms used to describe the arrangement of the uteri in nematodes; 5.) modified from illustration for *C. elegans* showing uterine and vulvular muscles.

20.) **Position of the Vulva**. Variation in the location of the vulva in plant parasitic nematode females.

21.) **Reproductive System**. Top right: the male- spicules, bursa types (leptoderan- bursa does not reach tail terminus, peloderan- bursa envelops the tail); Build-in 1: male tail (modified from illustration for *C. elegans*) showing the cloaca and gubernaculum; Bottom right: videos (*C. elegans*) showing mate-finding activity and copulation; Left: vulva of *C. elegans* and root-knot (RK) nematode; Build-in 2: egg development (*C. elegans*); eggs (stained red) in root tissue (lesion nematode); and hatching eggs (orange-unknown nematode (David Spears), green- *Heterodera schachtii*).

22.) **Nervous System**. Top right: modified from illustration for *C. elegans* sensory structures of male tail: B= bursa, R= bursal ray neurons, to NR= to nerve ring; Bottom right*: Contracaecum rudolphii*: S= sensillum; Bottom center: *Steinernema riobravis*; Bottom left: *Xiphinema americanum*: CS= cephalic sensillium (black arrows indicate amphid openings); Top left: NR= nerve ring; Top center: *C. elegans* male tail; Build-ins: 1.) (top) anterior of *Laxus oneistus* (A=amphid opening); (bottom) phasmid opening of *Scutellonema brachyurum* (hematoxylin-stained); *2*.) head regions of (right to left) *Rotylenchus* sp.; *Dolichodorus* sp. and *Neopsilenchus* sp. (from K.B. Nguyen); 3.) A= amphid and illustration of the complexity of this sensory organ.

23.) **Digestive System**. Right center: anterior of a second stage juvenile of *Heterodera glycines* in 'Lee' soybean root (B.Y. Endo); Build-ins: 1.) anterior of nematode showing the stylet orifice; 2.) illustration of stylet musculature and connection with the esophageal lumen (green shows connection of the stylet with the esophageal lumen); 3.) anterior of *Hoplolaimus galeatus*; 4.) further along the esophagus (*H. galeatus*); 5.) illustration of the anterior of a nematode to the junction between the esophagus and the intestine; 6.) C= cardium of *Tylenchorhynchus claytoni* (BBE = basal bulb of esophagus, INT= intestine); 7.) DS= digestive system, dots trace the beginning to the end of the digestive system.

24.) **Excretory/Secretory System**. Top left: illustration (modified) of *Plectus* sp.; Top right: the excretory pore of *C. elegans*; Build-in: location of the excretory pore (exc. pore) and excretory gland (gland) on the illustration.

25.) **Muscular System**. Elements of the muscular system (red lettering) in mid (A) and anterior (B) portions of the nematode body. Modified from illustrations in The Atlas of *C. elegans* Anatomy (Altun, Z.F., R. Lints and D.H. Hall, 2002-2006). NOTE: The website www.wormatlas.org has a wealth of excellent information that anyone interested in nematodes of any kind would benefit from reviewing. Build-ins: 1.) reflexed ovary shown in cross-section in figure A; 2.) functions of the hypodermis.

26.) **Nematode Habitats**. Ecto- and endoparasitic nematodes. Top left: lesion and sting (grayscale); Right: juveniles (red), males (green) and females (dark blue) of RK (root-knot), CN= cyst nematode. Bottom illustration modified from: Hesling, J.J. and H.R. Wallace, 1961. Observations on the biology of chrysanthemum eelworm *Aphelenchoides ritzemabosi* (Schwartz) Steiner in florists chrysanthemum. I. Spread of eelworm infestation. Annals of Applied Biology 49:195-209.

27.) **Parasitized Roots**. Top left: white females of soybean cyst nematode (SCN) on soybean (www.entm.purdue.edu); Top right: cyst juvenile hatching from egg (ucdnema.ucdavis.edu) and lesion nematode female; Bottom right: ring nematodes feeding on alfalfa roots; Bottom center: *Longidorus africanus* feeding on root tip (www.faculty.ucr.edu); Bottom left: sugarcane root systems with and without nematodes (nematode sizes exaggerated for emphasis); magnifying glass inset shows swollen root-knot and vermiform spiral nematode females near root

section. Build-ins: 1.) stained SCN juveniles in soybean root tissue (www.extension.missouri.edu); 2.) above-ground symptoms of damage on horticultural crops: A & B.) boxwood nursery field and plant, respectively (Alexandria, LA) infested with root-knot nematode; C.) peach trees declining due to dagger nematode (Clinton, LA); D.) declining golf turf due to lance and sting nematodes (Bastrop, LA); 3.) above-ground symptoms of damage on agronomic crops; A-C.) (LA) A.) *Meloidogyne incognita* infested corn field (left row treated with Telone and right row not treated); B.) *Rotylenchulus reniformis* infested cotton soil (foreground not treated, background treated with Telone); C.) cotton field infested with *M. incognita* and *R. reniformis*; D.) soybean field infested with *Heterodera glycines* (www.entm.purdue.edu).

28.) **Symptoms of Damage from Foliar (Above Ground) Nematodes.** Rice (Crowley, LA), anemone (www.ppdl.purdue.edu), phlox (plant-disease.ippc.orst.edu), wheat, alfalfa (www.agf.gov.bc.ca), coconut (nematology.ifas.ufl.edu), pine (www.oznet.ksu.edu); Build-ins: 1.) tulip (www.eppo.org); 2.) self-explanatory; 3.) a point to ponder!

29.) **Estimate of Yield Losses Caused by Plant Parasitic Nematodes**. Loss estimates range from a low of 6.3 percent for barley (left, life-sustaining crops) to a high of 20.6% for tomato (right, economically important crops). Globally, the value of these losses is estimated to exceed $77 billion U.S. dollars.

30.) **Nematode Movement and Dissemination**. Build-outs: 1.) crawling *C. elegans* (www.abac.edu); 2.) movement pattern, modified from Brusca, R.C. & J.G. Brusca. 1990. Invertebrates; 3.) humor, however an excellent reference is: Robinson, A.F., et al. 2005. Vertical distribution of *Rotylenchulus reniformis* in cotton fields. Journal of Nematology 37 (3): 265-271. Photographs: propagative stock (blog.agriculture.ph), tire (www.istockphoto.com), seed debris. Build-in: cartoon depicting the spread of nematodes such as is known for the potato cyst nematode, *Globodera rostochiensis,* via bird droppings. See also the work of Poinar and Yanoviak summarized on slide 94.

31.) **Nematode Sampling Methodology**. Modified from Zuckerman, Mai & Rhode. Build-in: a simple, but effective, message for producers (left image modified from one by G.L. Tylka).

32.) **Nematode Extraction Techniques**. Techniques (left top and bottom) illustrated include: the Baermann method (using funnels outside [usually for soil] or inside of a mist chamber [usually for root material], or a modification of the Baermann technique employing hardware cloth “sandwiched” between layers of PVC pipe [commonly 15-20 cm. in diameter] used for extraction from larger volumes of soil; and the semi-automatic elutriator (right top and bottom). Build-in: technician using the sugar-flotation centrifugation extraction technique.

33-36.) **Nematode Population Dynamics**. Information on slide 36 is modified from: Norton, D.C., 1978. Pp. 59-79 *In*, Ecology of Plant-Parasitic Nematodes. John Wiley & Sons, New York. Note: In an agricultural environment, the number

of genera comprising a community is usually, at most, 6-8. However, in a natural community the number of genera is frequently 30-60.

37.) **Nematode Action, Damage and Economic Thresholds**. Photographs: (left to right) cyst stage of *Heterodera glycines*, circular patch of stunted plants (soybean) "typical" of nematode injury, tomato roots galled by root-knot nematode and reniform female on root (cotton). Build-in: Nematode threshold information from other states. Terms: ET- defined on slide 37; DT (damage threshold)- the nematode level at which (relative to a nematode-free comparison) significant damage can be expected; AT (action threshold)- the nematode level at which some type of management tactic should be initiated. The table at the bottom provides current threshold (ET, DT, or AT) recommendations from the states listed and was supplied by: AR (T. Kirkpatrick), DE (R. Mulrooney), GA (R. Kemerait), IL (G. Noel & J. Bond), IA (G.L. Tylka), MS (G. Lawrence), SC (J. Mueller), TN (P. Donald & M. Newman) and VA (P. Phipps).

38.) **Nematode Management Tactics**. Top right: no-till cropping system (www.prebleswcd.com).

39.) **Nematicides**. Methyl bromide (entwew.clemson.edu), Temik (www.bayercropscience.cl), Furadan (www.sonti.cn). Build-ins: 1.) "hole" in the ozone layer of the atmosphere over Antarctica caused by human-produced compounds that release chlorine and bromine gases (www.NASA.gov); 2.) information about non-fumigant nematicides. Note: As of 11/2009, discontinued fumigants include: Meth-O-Gas, Brom-O-Gas, Terr-O-Gas and Vorlex. Discontinued Non-fumigants include Dasnit and Nemacur. Also note that nematacides formulated as seed treatments include Avicta Complete Cotton and Avicta Complete Corn (both produced by Syngenta) and AERIS Seed-Applied Insecticide/Nematicide (produced by Bayer Cropscience).

40.) **New Tactics for Nematode Management**. Photographs: (left to right) applying a low-dose nematicide as an at-planting, in-furrow spray; satellite (www.fcc.gov) and GPS technology in agriculture, nematode-trapping fungus (*Arthrobotrys* sp.).

41.) **Reduced Rate Nematicide, an Example**. (from current research in the nematology program of the LSU AgCenter with an experimental colloid). Build-ins: 1.) illustration of the 12 crops tested in trials in Louisiana; 2.) the greatest and most consistent yield response has been with cotton (3-acre field trials); 3.) self-explanatory; 4.) methods of application evaluated with this product (in all trials, a rate of 10 GPA of a 1% solution was employed, "transplant dip" treatments were for 8 seconds in a 1% solution.).

42.) **Site Specific Farming, an Example**. The use of GPS technology to document the exact location of a specific agricultural field ( from Google Earth). Build-in: a 100-acre cotton field in north Louisiana where the soil is co-infested with high levels of root-knot (*Meloidogyne incognita*) and reniform (*Rotylenchulus reniformis*) nematodes.

43.) **Site Specific Farming 2**. Top left: photograph and video, Build-in 1.) of a Veris 3100 soil electrical conductivity mapping system; Bottom left: diagram of the Veris 3100 instrument and explanation (right) of how it is used to determine soil electrical conductivity (EC). Other Build-ins: 2.) field map produced following delineation and mapping of EC values; 3.) establishment of verification strips; 4.) indicators of areas of the field that responded and failed to respond to treatment with a nematicide (Telone) in year 1.

44.) **Site Specific Farming 3**. A management zone map indicating areas of the field that should and should not be treated with a nematicide in year 2. Build-in: fumigant application implement.

45.) **Site Specific Farming 4**. The bottom line.

46.) **Site Specific Farming 5**. Software and hardware available to agricultural producers.

47.) **Nematode Parasites: *Pasteuria penetrans***. Build out: information about *P. penetrans*. Life cycle (modified from that at www.pasteuriabio.com), electron micrographs (respectively, grayscale and colored electron micrographs from www.rothamsted.ac.uk by B. Kerry & K. Davies), *P. penetrans* endospore; Build-ins: 1.) (left) a low magnification photograph of a female sting nematode, (right) the anterior portion of a sting nematode female; 2.) colorized sting nematode and attached endospores (orange) of *P. penetrans*; 3.) information about other species of *Pasteuria* and the nematodes that they parasitize.

48.) **Nematode Parasites: Fungi**. Top left: Electron micrograph of a nematode trapped by *Arthrobotrys* sp.; Top right: trapping loops of *Dactylaria brochopaga* (www.iwf.de); Bottom: (left and right) RK (root- knot) eggs parasitized by fungi; Center: J4 of SCN (soybean cyst nematode) parasitized by ARF fungus.

49.) **Expanded Phenetic Grouping of Nematoda. (**Mai & Lyon, 1975**)**. DEGO (dorsal esophageal gland orifice). Top right: (center) male of *Tylenchorhynchus martini*, (left) female of *Gracilacus* sp., (right) female of *Paratylenchus* sp.; Bottom right: female of *Hemicriconemoides* sp.; Left: (top to bottom, respectively) females of *Helicotylenchus* sp., *Hoplolaimus* sp. and *Pratylenchus* sp.

50.) **Important Genera of Tylenchida**. Self-explanatory.

51.) **Evolution of Parasitism in Secernentea**. Photos and credits (except top left [*H. galeatus* infecting grass roots]) identified earlier.

52.) **Illustration of Modes of Parasitism**. Modified from the artistry of R.P. Esser. Self-explanatory.

53-67.) **Key to Genera of Plant Parasitic Nematodes**. Some images included in this section were initially scanned at high resolution from the Pictorial Key to Genera of Plant Parasitic Nematodes by W.F. Mai and H. H. Lyon (see Acknowledgements). Where quality was lacking (to the eye of the first author),

original literature was obtained, images scanned and used with the "layers" and "image adjustments" functions of Photoshop CS3 to produce the modified images used in this section of the presentation. Note: Pop-up images by couplets serve EITHER to clarify a character description OR to illustrate a genus (red line connecting photograph or illustration to genus name).

68.) **Comparison of Common Nematode Genera**. Where there is significant variation among species in a genus, as for example the cyst life stage of *Heterodera* species, an "average" size is depicted. Also, aside from the plant parasitic nematodes, a free-living nematode (*Rhabditis* spp.) is included in the lower left section of the circle.

69.) **Individual Nematode Genera of Greatest Economic Importance or Potential**. Self-explanatory. Photographs: Top right: anhydrobiotic juvenile of *Rotylenchulus reniformis*; Center: molting juvenile of sting nematode, egg of *R. reniformis*; Bottom right: tail of male of *Bursaphelenchus xylophilus*; Bottom center: anterior of lance nematode female and female lesion nematode; Bottom left: anterior of female of *Hoplolaimus galeatus*; Top left: juvenile of root-knot nematode.

70.) **Reniform Nematode**. Photographs: Top: anhydrobotic juvenile, esophagus of juvenile; Middle row: (left to right) female on root (LM= light micrograph), female on root (EM= electron micrograph), infective fourth-stage juvenile on root, male; Bottom row: (left to right) females on root, soybean and cotton fields infested with *R. reniformis*, egg mass; Build-ins: 1.) stained and non-stained egg masses; 2.) host range; 3.) video.

71.) **Soybean Cyst Nematode**. Photographs: A.) a soybean field infested with the cyst nematode; B.) "yellow females" on soybean roots; C.) cyst with internal eggs visible; D.) "swollen" stages in the development of a cyst (white to brown); E.) soybean root tissue containing stained juveniles; F.) juvenile; G.) individual sperm cell of male; H.) second stage juvenile hatching from egg. Build-ins: 1.) cartoon showing the development of adults and cysts (modified, original source unknown); 2.) (left) female and male, (right) egg-filled cyst; 3.) females of the nematode compared with the size of a nodule on soybean; 4.) video (still photo comparing sizes of cyst females and nodules on roots from G. L. Tylka); 5.) egg-filled cysts of *Heterodera glycines* (100X).

72.) **Root-Knot Nematode**. Photographs: A.) a swollen endoparasitic female and external egg mass (both stained) in galled root tissue; B.) root tissue containing stained juveniles; C.) progressive (left to right) life stages (except egg) of the nematode; D.) galled tomato roots; E.) plants employed in the host differential assay (for the identification of "common" species and races…see slide 73); F.) galled carrot roots. Build-ins: 1.) (left) female dissected from fresh cucumber tissue, (right) female and egg mass from *Chenopodium* (E.C. Bernard); 2.) root-knot (*M. incognita*) infested field of soybeans near Alexandria, LA and galled root system; 3.) video.

73.) **Root-Knot Host Differential Assay**. This assay was developed by J. N. Sasser in 1954 and has been of immense value to the science of nematology. A plus (+) value indicates a susceptible host and a minus (-) value indicates a resistant host. Build-in: in addition to the host differential assay, perineal pattern morphology of root knot females (top center) is employed to distinguish between *Meloidogyne hapla* (Mh), *M. javanica* (Mj), *M. incognita* (Mi) and *M. arenaria* (Ma). In recent years, the use of esterase phenotypes (bottom center) is also employed as a further method of species confirmation.

74.) **Lesion Nematode**. Build-out: lesion nematode feeding in root tissue; Upper left: lesion nematode model (available from Mactode Publications); Lower right: female; Build-in: self-explanatory.

75.) **Sting Nematode**. Self explanatory. Lower left: sting nematode damage on strawberry; Build-in: self-explanatory.

76.) **Pine Wilt Disease 1**. Build-outs: Pine wilt symptoms on individual trees: 1- Louisiana, 1981; 2- Portugal, 1995 collecting pine wood samples near Setubal, PT (observations and collections made by E.C. McGawley while on a Fulbright sabbatical in Portugal first introduced agriculture and forestry personnel to this disease and made them aware of the likely presence of *Bursaphelenchus xylophilus* in the country); 3- Japan, 2008; 4- Pine wilt symptoms in a pine forest in Japan; Photographs: Top row: left- *M. alternatus* (L.D. Dwinell), cerambycid beetle on pine needles and log showing signs of infestation with blue-stain fungi (www.forestryimages.org) and cross section of *M. caroliniensis* trachea packed with juveniles of *B. xylophilus*; middle- Japanese black pines that are very susceptible to *B. xylophilus*; right- pine pallets, a likely vehicle for dissemination of the nematode; Bottom row: left- pine logs showing discoloration indicative of infestation with blue-stain fungi; middle- tail of male of *B. xylophilus* with characteristic “rose-thorn” shaped spicule (L.D. Dwinell); right- female of *B. xylophilus* (www.metla.fi). Pine wilt disease cycle in center of frame is modified from www.forestresearch.gov.uk.

77.) **Pine Wilt Disease 2**. Photographs: Top left: cultures of *G. virens* infested with *B. xylophilus* showing reduced growth and lack of sporulation (left) and similarly aged cultures sporulating in the absence of the nematode (right); the frame behind this shows the development of nematode-infested cultures over 12 days; Top right: culture of *G. virens* with characteristic “bowling pin” shaped phialides; Bottom left: *B. xylophilus* (respectively) male tail (low and high magnifications, respectively), juveniles of the nematode feeding on mycelium of *G. virens*, esophagus and vulva of female and line drawings of adults of the nematode; Bottom right: slash and loblolly pine seedlings used in inoculation studies conducted at LSU.

78.) **Lance Nematode**. Self explanatory.

79-80.) **Nematode Disease Complexes**. A good reference on this subject is: Sikora, R.A. and W.W. Carter, 1987. Nematode Interactions with Fungal and

Bacterial Pathogens – Fact or Fantasy. Pp. 307-312 *In*: Vistas on Nematology, J.A. Veech and D.W. Dickson, Eds., E.O. Painter Printing Co.

81.) **Nematode-Fungus Complexes**. Photographs: Left: (top to bottom) cultures of *Sclerotium rolfsii* (www.bspp.org.uk), *Fusarium oxysporum*, and soybean root infected with *Rhizoctonia solani*; Top: (left to right respectively) juveniles (www.rennes.inra.fr) of *Heterodera schachtii* and females of *H. glycines*, female and egg mass of root knot nematode, stained juveniles of *H. glycines*, fungal mycelium growing from plant tissue; Build-ins: 1.) an example of additivity: strawberry yield data are cumulative over the period 1989-1991. For other details, consult the Journal of Nematology citation indicated; 2.) an example of synergism. For other details, consult the Journal of Nematology citation indicated; 3.) an example of antagonism. For other details, consult the Nematropica citation indicated.

82.) **Nematode-Bacterium Complexes**. Photographs: Top: culture of *Ralstonia solanacearum* (www.cals.ncsu.edu); Bottom: female of *Aphelenchoides ritzemabosi* (modified); Build-ins: 1.) sections through nematode-parasitized and healthy soybean root nodules (www.micro.biol.ethz.ch); 2.) colorized electron micrograph of *Rhodococcus fascians* (www.mikrobenscout.de).

83.) **Annual Ryegrass Toxicity**. Build-out: sheep affected by ARGT. Photographs: ryegrass plant (members.iinet.net.au), ryegrass seed, anhydrobiotic nematodes (www.invasive.org).

84.) **Nematode-Virus associations**. Photographs: leaf symptoms (www.agf.gov.bc.ca); electron micrograph (www.ncbi.nlm.nih.gov). Arrows on illustrations (modified) of nematode esophagi indicate areas of greatest virus retention.

85.) **Nematode-Nematode Interactions 1**. Build-out: general information relative to studies of nematode-nematode interactions. Introduction to and citation for the DeWit replacement series methodology.

86.) **Nematode-Nematode Interactions 2**. Application of the DeWit replacement series for the evaluation of the interaction between root-knot and reniform nematodes. For other details, consult the Journal of Nematology citation indicated.

87.) **Nematode-Weed Interactions**. An example: in this work, it was demonstrated that root leachates from the weeds morningglory (MG), hemp sesbania (HS) and Johnsongrass (JG) inhibit reproduction of *Rotylenchulus reniformis* on cotton (C). The top photograph shows the experimental setup in which root leachates were collected from each of the weeds (hanging baskets used as controls (front) contained only sterile Perlite growing medium). The table on the right is reniform nematode population data averaged and analyzed over two trials. Photographs at the bottom illustrate the filtration of root leachates and the evaluation of their influence on hatch of eggs of *R. reniformis.* Build-ins: 1.)

data showing the numbers of undifferentiated, 8-16 cell and fully developed eggs and hatched juveniles (Y-axis) over a period of ten days (X-axis) of incubation in cell wells containing root leachate and control suspensions. Leachates from all three weeds reduced the rate of egg development. For further details, consult the Nematropica citations indicated; 2.) more common nematode-weed relationship in agriculture; 3.) key points regarding nematode-weed relationships in agriculture; 4.) weedy soybean field (top-www.extension.iastate.edu) and photo of female of SCN on purple deadnettle (E. Creech, Purdue University). Also, a current reference is: Johnson, W. G., Creech, J. E., and Mock, V. A. 2008. Role of winter annual weeds as alternative hosts for soybean cyst nematode. Online. Crop Management doi:10.1094/CM-2008-0701-01-RV.

88.) **Nematode-Insect-Fungus Interactions**. In this study, the stem canker fungus (DPC) caused reductions in the numbers of juveniles of SCN (soybean cyst nematode) juveniles present in root tissue. Conversely, defoliation by the SBL (soybean looper, *Pseudoplusia includens*) resulted in significant increases in the numbers of juveniles present in roots. Overall, the effects of these three pathogens on plant growth and each other were **additive**. For other details, consult the Journal of Nematology citation indicated.

89.) **Entomogenous Nematodes 1**. Build-outs: 1.) taxonomic position of most entomogenous nematode species; 2.) esophagus of nematodes in the order Rhabditida; Build-ins: 1.) photographs of *Heterorhabditis bacteriophora* (www.bio-control.nl) and *Steinernema carpocapsae* (www.db.uac.pt); 2.) intestine of *S. carpocapsae* showing resident *Xenorhabdus nematophilus* (www.uconn.edu).

90.) **Entomogenous Nematodes 2**. Life cycle of *Photorhabdus luminescens* (curiosidadesdelamicrobiologia.blogspot.com); Photographs: Left: nematodes burst forth from an insect cadaver; Right: caterpillars (*Manduca sexta*) infected with *P. luminescens* and glowing (www.nature.com); Build-in: *Photorhabdus luminescens* (www.sci.muni.cz). *Photorhabdus* means glowing rods; they are the only known terrestrial bioluminescent bacteria.

91.) **Entomogenous Nematodes 3**. Photographs: A.) mermithid nematode emerging from a fire ant (S. Porter); B.) nematodes emerging from wax moth (*Galleria mellonella*); C.) grass shrimp infected with nematode (mygrassshrimp.googlepages.com); D.) juvenile of the genus *Heydenius* emerging from a winged male ant of the genus *Prenolepis*. This specimen is preserved in Baltic amber approximately 40 million years old (G. Poinoir, 2002); E.) nematodes attacking a termite (bexar-tx.tamu.edu); F.) juvenile *Romanomermis culicivorax* emerging from a mosquito larva (University of Nebraska, Lincoln Dept. of Entomology).

92.) **Entomogenous Nematodes 4**. Photographs: A.) fungus gnat larva infected with *Steinernema feltiae* (www.omafra.gov.on.ca); B.) grub infected with *Heterorhabditis bacteriophora* (www.yardscaping.org); C.) grasshopper infected with *Mermis nigrescens*; D.) mosquito larvae infected with *R. culicivorax*; Build-ins: 1.) *Psammomermis* sp. (M. Hodda and www.csiro.gov.au); 2.) Skeeter Doom

package guaranteed to contain 500 mixed life stages of *Reesimermis nielseni* per gram of content.

93.) **Entomogenous Nematodes 5**. Commercial entomogenous nematode products. Self-explanatory.

94.) **Entomogenous Nematodes 6**. The relationship between *Myrmeconema neotropicum* and *Cephalotes atratus*. Self-explanatory.

95.) **Molecular diagnostics:** Title slide

96.) **Why use molecular methods?**

97.) **Molecular diagnostics** includes biochemical methods, DNA-based methods, and genomics methods.

98.) **Biochemical methods**: the most common biochemical method used for nematode diagnostics is the electrophoretic separation and analysis of enzyme isozyme patterns. These include esterase, malate dehydrogenase, and others. Often separated using a PHAST gel system (Pharmacia, Inc. may be defunct). Limitations-must have young females; variants seen within species; good method for tropical RKN identification.

99.) **DESS**: a versatile preserative for nematode PCR (described in detail in Yoder et al., *Nematology*, 2006, Vol. 8(3), 367-376). DESS= **D**MSO, **E**DTA, **S**aturated **S**alt) 0.25M disodium EDTA pH 8.0; 20% Dimethyl sulphoxide; NaCl saturated Preserves nematode morphology; inactivates nucleases that degrade DNAleft image = ; right image = ; courtesy Paul DeLey.

100.) **vCenema** movie clips courtesy Paul De Ley, Luis Mundo and Manuel Mundo. *Pratylenchus penetrans* gland bulb region and ovary tip region.

101.) **DNA based methods**: all specimen types are amenable to molecular analysis; however, cysts and eggs may require extra effort to break them open to release the DNA. The availability of specimens in many routine diagnostic situations is often limited to a few juveniles, so it is vitally important to develop methods of DNA extraction and molecular analysis that will be successful with single nematodes.

102.) **Preparation of nematodes for molecular analysis**; Nematode cuticles may be tough, so physical disruption is often necessary; chemicals alone are often inadequate or harsh (such as NaOH, which must be neutralized before the template can be used in PCR). While PCR can be performed with a relatively crude extract obtained from a single specimen, bulk nematodes generally give a more favorable DNA yield and purity when a commercial kit for DNA preparation is employed.

103.) **Downstream molecular analysis**: Polymerase chain reaction employs thermostable DNA polymerase (Taq polymerase) to synthesize copies of the target DNA, resulting in an exponential increase in the amount of DNA.

104.) **A closer look at PCR methods:** The following slides show the components of a typical PCR reaction and highlight ways to avoid contamination and other pitfalls. This photograph shows a sterile hood used as a PCR workspace, with the arrangement of equipment and supplies needed for reaction setup.

105.) **PCR hygiene:** Cleanliness is of utmost importance for PCR, but there are several steps one can take to reduce the possibility of contamination or degradation of reagents. DNA extracted from "nematode smash" is not difficult, but the resulting DNA is not pure, and nucleases present in the worm can be active at room temperature. Keeping extracts and PCR kit components cold can help prevent DNA degradation. Aerosol barrier tips can help prevent pipettors from getting contaminated with DNA, which could carry over from one experiment to another. It is always easier to throw away a small aliquot of a reagent than to spend weeks figuring out what went bad. Dividing Taq buffer, nucleotides, etc. into small aliquots prevents excessive freeze-thaw cycles that degrade reagent performance. Physical separation of pre- and post-PCR analysis helps limit the possibility of cross contamination.

106.) **Post-PCR:** Photographs in buildouts: 1) Middle, agarose is weighed; left, agarose gel solution is heated to boiling in a microwave oven; right, cooled agarose is poured into gel casting tray. 2) Left, electrophoresis running buffer is mixed on a stir plate; right, running buffer is poured into the gel box. 3) Left, 1% loading dye solution is added to each sample; right, samples are loaded into wells. 4) Left, electrical current is passed through the gel; right, tracking dye is used to follow progress of the electrophoresis. 5) Left, agarose gel soaking in ethidium bromide stain; right, Alpha Imager system used for digital documentation of gel images. Every lab has its own equipment and workflow habits, but these are typical steps for analyzing PCR reactions. When weighing agarose, it's a good idea to use a spatula for scooping; remember to discard excess agarose following appropriate waste disposal regulations (do not put it back in the bottle). Avoid dust when preparing agarose solutions (particles absorb UV light and make gels look messy). Allow molten agarose to cool some before pouring to avoid warping your gel trays. Modern imaging equipment makes archiving gel images much easier, but dark hood camera adapters that fit over a UV light box are inexpensive and easy to use.

107.) **Typical gel result from PCR amplification:** Photograph: gel electrophoresis of PCR reaction products. Consider how big of a gel you will need and what percentage of agarose will allow the best separation of the expected PCR products (typically, the smaller the product length in bp, the higher % agarose you need). Make sure to use big enough gel combs to accommodate the sample volume, pour your gel deep enough, and don't forget to leave room for your controls and DNA size markers. Gel bands can then be excised with a sterile blade, purified from the agarose, and used for downstream applications such as

direct sequencing, restriction digests, or cloning. Just don't forget your eye protection when working over a UV light source!

108.) **Molecular markers commonly used for nematode identification-1**: ribosomal genes are the most common target gene for nematode diagnostic PCR. The multi-copy arrangement of ribosomal gene arrays provides ample target even from single nematodes. Low variation amongst rDNA arrays within individuals often exists due to a process of homogenization known as concerted evolution, whereby the individual rDNA copies do not evolve independently from one another. Consequently, little variation in rDNA genes within a single nematode or population will be observed. Of course, there are exceptions, so when multiple sequence variants are observed, the process of homogenization may be incomplete.

109.) **Molecular markers commonly used for nematode identification-2**: The ITS rDNA is comprised of the internal transcribed spacer region between the 18S and 5.8S coding regions (ITS1) and between 5.8S gene and the 28S rDNA (ITS2). Due to the high level of sequence conservation in the coding regions that flank the ITS, a single universal primer set (such as TW81 and AB28) can amplify this region from the vast majority of plant-parasitic and free-living nematodes. The ITS rDNA is arguably the most commonly amplified, most useful diagnostic marker for the plant-parasitic nematodes.

110.) **Molecular markers commonly used for nematode identification-3**: 18S rDNA, also known as the small subunit ribosomal RNA (SSU). While this marker is more often used for phylogenetic analysis, it has shown some utility for identification of species in understudied genera such as for survey samples from terrestrial or marine environments.

111.) **Molecular markers commonly used for nematode identification-4**: 28S rDNA, also known as the large subunit ribosomal RNA (LSU). 28S structure domains D2 and D3 are most frequently used for nematode sequence analysis and identification. The secondary structure prediction based on loops and hairpin domains may be used to inform DNA important or conserved sequences. These structures can aid in alignment of sequences for phylogenetic analysis. Figures A and B from Subbotin, S.A., Ragsdale, E.J., Mullens, T., Roberts, P.A., Mundo-Ocampo, M., Baldwin, J.G., A phylogenetic framework for root lesion nematodes of the genus Pratylenchus (Nematoda): evidence from18S and D2-D3 expansion segments of 28S ribosomal RNA genes and morphological characters, Molecular Phylogenetics and Evolution (2008), doi: 10.1016/j.ympev.2008.04.028

112.) **Molecular markers commonly used for nematode identification-5**: IGS rDNA denotes the intergenic spacer region between the 28S gene and the 5S ribosomal subunit (IGS1) and the space between 5S and 18S (IGS2). This marker has shown utility for discrimination of root-knot nematode species, particularly *Meloidogyne mayaguensis*.

113.) **Molecular markers commonly used for nematode identification-6**: mitochondrial DNA markers. Comprises the interval between the cytochrome oxidase II gene, inclusive of the tRNA His gene, and ending in the 16S gene. Commonly used for discrimination of root-knot nematode species. Size of the PCR product or restriction fragment length polymorphisms are also diagnostic for some species.

114.) **Restriction fragment length polymorphism:** PCR products are digested with restriction enzymes, resulting in patterns of fragments that can be analyzed by gel electrophoresis. This method has been used for a wide range of plant parasitic nematodes and is relatively inexpensive and simple to perform.

115.) **Compare unknowns to reference species controls:** A key factor in the successful application of RFLP for nematode diagnostics is having a reliable panel of reference species to compare with unknown samples.

116.) **Real-time PCR:** A major benefit of real-time PCR is its versatility; however, as with any PCR assay, demonstration of sensitivity and specificity are key. Real-time PCR requires specialized instrumentation relative to conventional PCR, and the cost of reagents and equipment maintenance can be limiting. Proper training in experimental design and interpretation of the data are vita considerations.

117.) **Multiplex PCR:** This approach is most often comprised of species-specific primers used in combination to simultaneously detect or discriminate nematode species. One of the most well known applications of this method is the ITS rDNA based multiplex PCR for detection of potato cyst nematodes *Globodera pallida* and *G. rostochiensis.* The method was recently modified to also allow detection of *G. tabacum*. This method should be used cautiously when there is the possibility of intraspecific variation in the chosen marker. Validation of new assays against a wide geographic range of populations and control species is always desirable.

118.) **Section credits:** Self-explanatory.

119.) **Buffered and Non-Buffered Ecosystems**. Buffered ecosystem-a group of interdependent plants and animals inhabiting the same region or area and interacting with each other through food and other relationships in which all acting influences are canceled by others, resulting in a stable, balanced, or unchanging system in which no one species predominates.

120.) **Students: the Most Important Product**. Top row (L to R) C. Overstreet, K.L. Winchell, K.C. Hadden, J.P. Bond and I. Wenefrieda; Middle row (L to R) E. Wosula, A. Sankaralingam and M.J. Pontif; Bottom row (L to R) F. Garces, S. R. Stetina and J. Bruce. Build-in: L - M. Parish; R – A. Staszkiewicz.

121.) **Closing**. Self-explanatory.

122.) **Acknowledgements**. We sincerely hope that the credits here in the syllabus and on slide 98 provide proper acknowledgement of individuals and websites from which materials used in this presentation were collected. Materials from the authors of this presentation are not credited herein. Observers and users of this material are encouraged to contact the authors if any non-credited materials are observed so that they can be added where appropriate.

123.) **Additional note**. Self-explanatory.

Author email contacts are: emcgawley@agctr.lsu.edu, coverstreet@agcenter.lsu.edu, mpontif@agcenter.lsu.edu, and andrea.skantar@ars.usda.gov