Quantifying nematode suppressive potential of the *Crotalaria juncea* cultivar, 'Crescent sun'

Background.

Crotalaria juncea, or sunn hemp, is a legume often grown as a cover crop in tropical regions to provide nutrients to crops (Rotar and Joy, 1983). Previous research (Wang 2002) has shown that *Crotalaria* can suppress nematodes such as root knot nematode (*Meloidogyne* spp.) and soybean cyst nematode (*Heterodera glycines*), however, it may also act as a host to other pest nematodes such as lesion nematode (*Pratylenchus* spp) and ring nematode (*Criconemella* spp). If *Crotelaria* can be shown to suppress the nematodes of concern in California agriculture (*Meloidogyne, Pratylenchus vulnus, Criconemella xenoplax*) then this could be used to justify its' use as a cover crop to growers. The following experiments will focus on one cultivar, 'Crescent sun', which has a well-established certified seed system that could meet potential seed demand from growers.

Objectives. 1) To quantify the potential of the *Crotelaria* variety, 'Crescent sun', to suppress three species of plant parasitic nematodes in greenhouse trials. **2)** To compare the ability of 'Crescent sun' to suppress *M. incognita* in field microplots.

Strategy. To determine if 'Crescent sun' will act as a host, greenhouse trials will compare the ability of three species of nematodes (*Meloidogyne incognita, Pratylenchus vulnus, Criconemella xenoplax*) to survive and reproduce on the plant. This will provide information on what crops and nematode pest systems the variety is most suited to. Follow up field microplot trials will evaluate the ability of cover cropping with 'Crescent sun' to reduce damage in one pest crop combination, for example *M. incognita* and tomatoes.

Experimental Design.

Phase I: Greenhouse experiment

Beginning in a simplified greenhouse environment will enable us to test several species of nematodes simultaneously in a small amount of space. This is important because it is likely based on the scientific literature that the cultivar will be susceptible to *Pratylenchus* spp. and *Criconemella* spp.

In this experiment, we will test the ability of three nematode species (*Meloidogyne incognita, Pratylenchus vulnus, Criconemella xenoplax*) to infect the 'Crescent sun' variety. Seeds will be sown into 32 oz pots filled with sandy soil and once plants have established, they will each be inoculated with 100 individual nematodes. *M. incognita* will come from cultures of maintained in the greenhouse on the tomato (*Solanum lycopersicum*) cultivar 'Rutgers'. Nematode eggs will be harvested using the NaOCl method and infective juveniles collected by Baermann funnel (Hussey and Barker, 1973). *P. vulnus* and *C. xenoplax* will come from sterile grape root tissue cultures maintained by the Hodson lab. Plants will be watered and fertilized in the greenhouse daily and nematodes will be allowed to reproduce for eight weeks after which plants will be harvested and the number of nematodes present in the soil quantified.

There will be 6 treatments, 3 treatments with each of the nematode species (*M. incognita, P. vulnus, C. xenoplax*) growing on 'Crescent sun', and 3 treatments with the nematodes growing on a susceptible

host, such as melon. There will be 5 replicates of each treatment for a total of 30 pots. The experiment will be repeated once.

Timeline: September – December 2020

Phase II: Field experiment

We expect that 'Crescent sun' will be suppressive to root knot nematodes. Although it is possible that 'Crescent sun' suppresses all three species, the following outlines an experiment only for the *M. incognita*/tomato pest-crop complex. Other combinations, such as *P. vulnus* on almonds, could also be alternatively tested.

While the greenhouse experiment is being conducted, in the summer of 2019 we will establish populations of *M. incognita* growing on tomatoes in microplots. In the fall, tomatoes will be removed and carrots planted to maintain nematode populations over the winter. In March/April of the spring of 2020 'Crescent sun' will be planted and allowed to grow for 8 weeks, after which the residue will be incorporated into the soil and tomatoes again planted.

Each experimental plot unit will consist of three, 55 gallon food grade plastic barrels placed in the ground so that only eight inches is visible above the soil surface. Each barrel will have drainage holes and be filled with four inches of gravel on the bottom and washed 60 mesh sand on the top. These treatment plots will be replicated across four blocks, with each block having 3 barrels of each treatment, arranged in a randomized complete block design. There will be three treatments, 1) 'Crescent sun' winter cover crop 2) Bare soil over the winter and 3) Vydate, a conventional nematicide, applied before planting in the spring, for a total of 36 barrles (3 treatments x 4 replicates x 3 barells/replicate).

Tomatoes will be started from seed in the laboratory in two inch pots filled with 50 percent sand and 50 percent UC mix. After two weeks when they have reached approximately 10 cm height, tomatoes will be planted in the field. Three tomatoes will be planted in each microplot barrel and tomatoes will be thinned to one tomato plant per barrel after one week. Tomatoes will be irrigated every other day by drip irrigation and fertilized as needed.

Nematodes will be inoculated into barrels one week after tomato planting. Nematode inoculum will be prepared by extracting eggs from roots of greenhouse-grown tomato ('Rutgers') with 1% NaOCl. Eggs will be hatched in glass Baermann funnels and IJs collected. Each barrel will be infested with approximately 10,000 IJs applied in 2.5 ml aliquots to two holes about 5-cm deep and 1-cm wide in the soil around each tomato plant. A second inoculation will occur in the fall to maintain infections on carrots, occurring 1 week after planting.

At harvest, in later summer 2021, tomatoes will be removed and weighed based on red, green and unmarketable categories. Plants will be cut at the soil surface and fresh aboveground biomass determined. A subsample of foliage will be taken back to the lab for determination of dry matter. Root systems will be removed from each barrel, transported to the lab and washed. The level of nematode infection will be assessed using the gall index 2 system, established by Hussey and Janssen (2002), where roots are evaluated on a scale of 0 to 5 based on the percentage of the root system with galls. In this system 0 = no galling; 1 = trace infection with a few small galls; 2 = $\leq$ 25% roots galled; 3 = 26 to 50%; 4 = 51 to 75%; and 5 = >75% roots galled. To further evaluate nematode resistance, roots will be weighed, and nematode eggs extracted to determine eggs/g of root tissue.

Timeline: June 2020 – September 2021

References.

Hussey RS, Janssen GJW. Root-knot nematode: Meloidogyne species. In: Starr JL, Cook R, Bridge J, editors. Plant Resistance to Parasitic Nematodes. Wallingford, UK: CAB International; 2002. pp. 43–70.

Rotar, P. P., and Joy, R. J. 1983. 'Tropic Sun' sunn hemp, Crotalaria juncia L. HITAHR Research Extension Series 036. University of Hawaii, Honolulu, HI.

Wang, K.-H., Sipes, B. S., and Schmitt, D. P. 2002. Crotalaria as a cover crop for nematode management: A review. Nematropica 32:35–57.

Budget.

Total = $15,043

Phase I: Greenhouse experiment: $6000 ($1000 per treatment). 'Crescent sun planting, maintenance and buildup of nematode cultures to reach desired infestation level, extracting nematodes from soil and counting under the microscope. Analysis of results and preparation of report.

Phase II: Field experiment: $9,043 ($2,261 per treatment)

Microplot maintenance, including water and fertilizer application, weeding, and mowing alleys.

Contracted labor. ~$500

Preparation of nematode inoculum and nematode application. Maintenance and buildup of nematode cultures to reach desired infestation level. Extraction of eggs from culture plants, hatching of eggs on Baermann funnels. Calculating nematodes inoculum levels, applying nematodes to the barrels.

Laboratory technician: 10% FTE: $4,923.

Tomato planting, cover crop planting, application of nematicides and tomato harvest. Analysis of data and preparation of report.

Professional Researcher 2% FTE: $2,162

Undergraduate assistants 1% FTE: $243.

Total: $2,405.

Quantifying galling on roots, weighing roots, extracting eggs from roots and quantifying eggs/g root tissue.

Undergraduate assistants: 5% FTE: $1,215.