THE CYST TECH® PROCESS

I. Overview of Cyst Nematode Problem

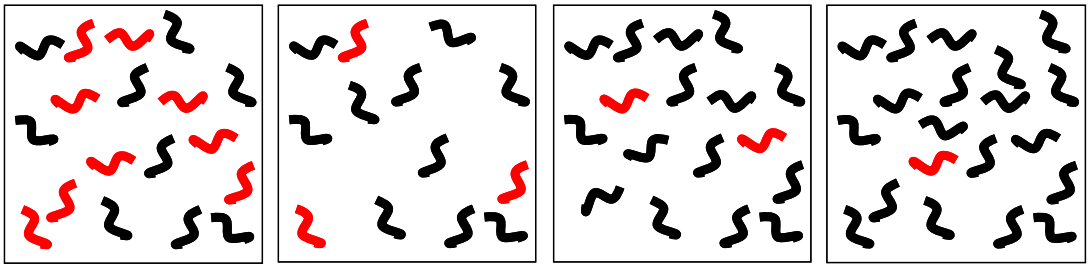
The soybean cyst nematode (Heterodera glycines) is a serious yield-limiting pest of soybeans. The nematode exhibits long-term survival through eggs in the soil and egg counts, and population densities of the nematodes in the soil build up each year that susceptible soybeans are grown. In fact, even the use of resistant SCN varieties of soybeans does not effectively control the nematodes in the long-term. A major problem with this pest is that severe yield losses (as high as 40%) have been reported in the absence of above ground symptoms in soybean fields.

Several sources of genetic resistance to SCN have been identified in soybeans, and the mechanism of the resistance appears to be a hypersensitive response. In this case the feeding of SCN juveniles on root causes the formation of a structure called a feeding sire, which supplies nutrients to the nematode. There are a total of 16 races of SCN known today. A few of the available resistant soybean lines are capable of reducing 3 to 4 different races, but leave the other races unharmed to reproduce, resulting in a "race shift". Race shifts can occur in a single year to a race that cannot be controlled with resistant lines. This makes resistant lines obsolete.

The different sources of genetic resistance to SCN that are available apparently give resistance to different races of SCN. This is a common occurrence in the relationship between host organisms and their pathogens, where genes for resistance in the host co-evolve with genes for virulence in the pathogen.

It has been accepted practice for sometime to use soybean lines carrying SCN resistant traits to avoid some of the yield losses associated with high levels of infestations of the nematodes (usually measured by egg counts per 100cc of soil in the field). However, it was quickly realized that the use of only one source of SCN resistant soybeans was only a short-term solution to the problem. It is now evident that SCN populations in the soil consist of soybean lines. In other words, one source of resistance will reduce feeding by one nematode race but not by the 16 and thus different sources of resistance will have different effect on different SCN races. This leads to a phenomenon (Figure 4) that has been labeled "race shifting" when soybean lines resistant SCN are planted over several seasons.

Figure 4: Soybean Cyst Nematode Race Shifting



Season 1	Season 2	Season 3	Season 4
(Plant Beans Res to)	(Plant Beans Res to)	(Plant Beans Res to)	(Plant Beans Res to)
Mixed Nematode Population	Reduced Population	Increase of Unaffected Race	New Race Dominates

As shown in Figure 4, repeated planting of soybeans resistant to SCN leads to a reduction in the nematode population for the next season, but planting the same beans sequentially over several seasons leads to a build up of the nematodes that are not affected by the source of resistance in the soybeans.

II. Overview of Mark Seed Research on Soybean Cyst Nematode

Research at Mark Seed on the SCN management problem began about 12 years ago. Since then, the ongoing demand for new soybean germplasm sources of resistance to the nematodes and a slow increase in the industry understanding of how to manage race shifts in the soil has led to a general acceptance of the need for rotation of susceptible and different resistant soybean lines. However, the problem has persisted and in fact is now perceived as growing worse. For example, over the years SCN populations have adapted to SCN-resistant soybean varieties to the extent that as many as 60-70% of nematode types can now attack a soybean variety being marketed as SCN-resistant, and it was recently noted that 84% of soybean fields are infected with SCN.

The breakthrough concept that has arisen from this research work is that specific combinations of soybean lines with differing race resistance and susceptibilities should be rotated rather than rotating pure lines with only one or two race resistant genotypes.

While the initial research was conducted through Mark Seed, extensive multi-year, multi-location field trials have been conducted with universities and commercial partners, and a large data set is available.

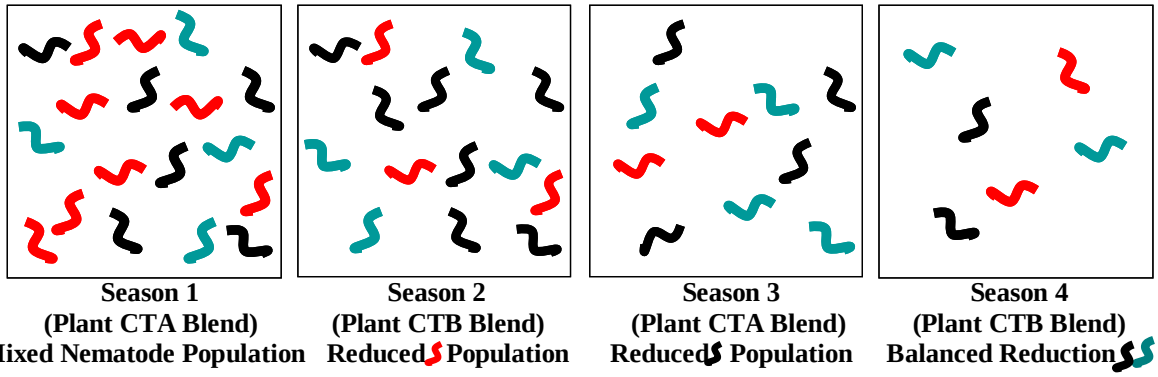
III. Explanation of the Cyst Tech® Process

Cyst Tech® rotations are designed with input on the SCN race types present in any given field environment, and sequential planting different combinations of soybean lines, the combinations being classified as CTA and CTB.

Combinations can vary in composition by the percentage of different susceptible and race specific resistant soybean lines, but the consistent rotation relies on CTA followed by CTB combinations. The selection of combination percentages is based on SCN race typing and on experience from season to season. The initial criterion by which the performance of the combinations is measured is based on the previous years' soybean types. There appears to be a good correlation between the reductions of SCN egg counts in the soil and progressive yield increases under CTA-CTB rotations.

Figure 5 below is a simplistic representation of the Cyst Tech® rotation effect on SCN populations during CTA-CTB rotations.

Figure 5: Cyst Tech® CTA & CTB Rotational Effectiveness



Season 1 (Plant CTA Blend) Mixed Nematode Population | **Season 2 (Plant CTB Blend) Reduced Population** | **Season 3 (Plant CTA Blend) Reduced Population** | **Season 4 (Plant CTB Blend) Balanced Reduction**

Note that CTA and CTB combinations can vary from season to season depending on SCN race frequency estimations. The exact mechanisms that cause the reductions in SCN populations are not completely clear, and may involve micro-environmental effects in the soil that inhibit SCN population growth through allelopathic interactions between different soybean lines. Certainly, the suggested move away from monoculture of single soybean lines towards combinations designed to control SCN populations and circumvent the problems of race shifting is a new option for the soybean grower.

Although more details of the system will be supplied to interested parties, the Cyst Tech® system can be summarized as follows:

1. **The previous soybean crop history will be used to determine whether CTA or CTB should be used first.**
2. **Selection and sequential planting of appropriate CTA and CTB combinations lead to a steady reduction of the total SCN population and eventual predominance of the race three.**
3. **It appears that the CTA varieties combat race shifting and CTB varieties control population densities.**

It has been a general observation that most field populations of SCN start out as race three, and then when the resistant soybean is used year after year, the nematode races that are able to reproduce on the genetically resistant variety increase. Thus, after some time, the repeated use of that specific kind of genetic resistance is no longer effective. Thus the effectiveness of the Cyst Tech® approach is based on its ability to maintain SCN race three multiple sources of resistance to SCN in the seed combinations, the basis of the technology is not resistance, but rather control through seed batches that are mixtures of seed lines with carefully calculated composition. The long-term application of the Cyst Tech® technology leads to large reductions in SCN egg counts in the soil and significant soybean yield increases.