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PROGRESS ON THE EARLY DETECTION OF RHIZOMANIA UTILIZING REMOTE SENSING

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2387 individual spectra were collected with an ASD FieldSpec Pro spectroradiometer and 804 digital photos were collected from 115 individual sugar beet plants at 20 sites weekly from July 11, 2003 through August 22, 2003 from plots located at Crookston, MN, Glyndon, MN, fields in production between Crookston, MN and Glyndon, MN, and potted plants at North Dakota State University in Fargo. One plant from each site was tested upon completion of data collection to determine if individual sites were Rhizomania positive or Rhizomania negative during the growing season. The spectra were then analyzed and aggregated for a database to determine the spectral characteristics of sugar beets in various stages of Rhizomania infection. Digital photos of individual sites collected on spectra collection dates will also be visually analyzed to determine the visual Rhizomania symptoms present at the time of spectra collection. If the spectral characteristics of Rhizomania infection can be determined, a method of early detection utilizing remote sensing will then be developed.
A project scheduled to include two growing seasons of data collection, direction of research for the 2004 growing season will be based upon the first year's findings.

OBJECTIVES

The objectives are to 1) determine the spectral characteristics of early Rhizomania infection in sugar beets
and 2) develop a method of early detection of Rhizomania infection in sugar beets utilizing remote sensing methods with a high degree of probability.

METHODOLOGY

Sugar beet spectra were collected at 3 sites from a University of Minnesota (U of M) plot at Crookston, MN, 3 sites from a North Dakota State University plot at Glyndon, MN, 20 potted plants on the North Dakota State University (NDSU) campus at Fargo, ND and 1 site each from 13 fields in production between Crookston and Glyndon with the cooperation of 3 producers. The spectra were collected with an Analytical Spectral Devices, Inc. (ASD) FieldSpec Pro spectroradiometer. The Field Spec Pro records a data point of representing the intensity of the energy reflected and emitted by a target at each nanometer (nm) between 400 nm and 2500 nm (Figure 1).
Digital photos were also collected at each site at the time of spectra collection as a reference for visual indication of Rhizomania infection. A wavelength check was performed on the Fieldspec Pro weekly with a Hg/Ar lamp by selecting 10 identification points between 546 and 1709 nm to ensure wavelength accuracy.

The collection site locations chosen in each plot or field were located near drainage ditches or road ditches if possible. Data collection started July 11 and heavy rains in the Red River Valley in June caused visually noticeable water stress in ditch areas after this heavy rainfall period. The hypothesis for site collection locations was that Rhizomania infection would likely start in the areas of visible water stress and move outwards as the growing season progressed. It would then be possible to record sugar beet spectra in increasing degrees of Rhizomania infection during the growing season. Therefore, the sites were located just outside of visually stressed areas, or in areas that appeared visually healthy but still adjacent to visibly stressed areas.

Each collection site consisted of 5 individual sugar beet plants arranged in identical patterns at each collection site. The 5 individual plants were identified as P 1-5 with 7" X 5" orange flags extending approximately 24" above the ground at each collection site. Plant 5 at each site was surrounded by Plant 1 on the north side, Plant 2 on the east side, Plant 3 on the south side and Plant 4 on the west side.

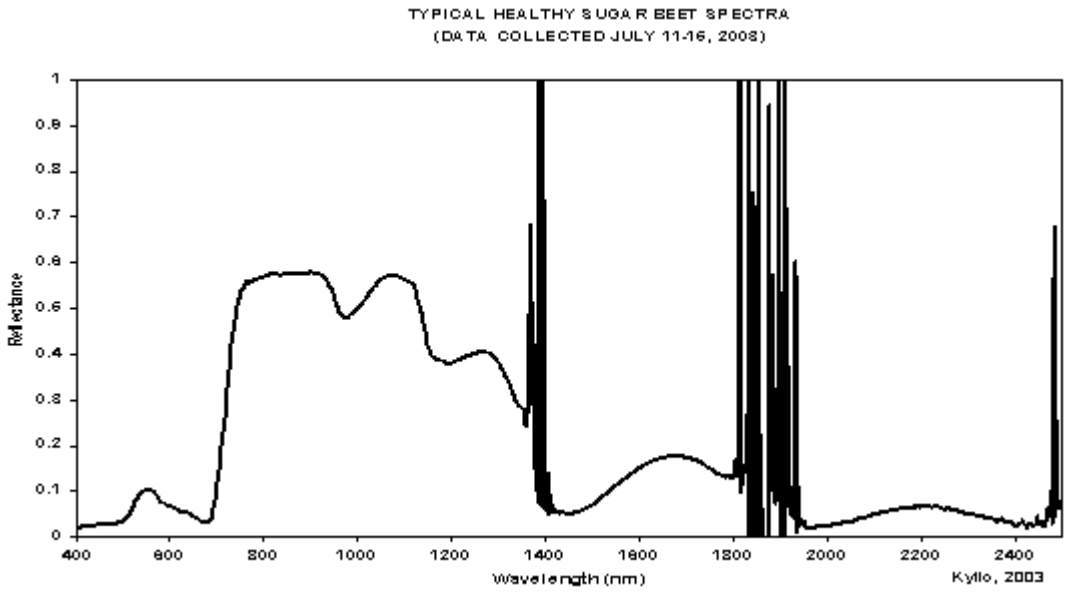

Figure 1. A spectra of a healthy sugar beet collected in the period of July 11-16, 2003. The data is obtained in text format, reformatted into Excel format, and charted in this manner. This chart is a collection of 2100 individual data points at each nanometer (nm) from 400 to 2500 nm representing the intensity and wavelength of energy recorded each data point. The features at 1400 nm and between 1800 and 2000 nm are attributed to water in the atmosphere and are typical.

The 20 plants at NDSU were identified as Plants 1A-Plants 5D to account for varietal differences. Data was collected identically at each site on each date, moving from Plant 1 to Plant 5 at each site and at each of the 20 plants at NDSU from Plant 1A-Plant 5D. It was felt that identical patterns at each collection site would result in more efficient data collection and greater ease of data analysis. Spectral data and digital photos were collected from each site weekly from July 11 through August 22, weather permitting. Data was only collected on clear, sunny days or on days of scattered clouds when breaks in clouds would allow data collection between clouds. Prior to the arrival at a data collection site, the FieldSpec Pro was turned on from 30–60 minutes to allow proper warming of internal electronics.

At a data collection site, an initial white reference and optimization were performed internally on the Fieldspec Pro using a spectralon disk prior to each collection, repeated within approximately 20 minutes during each period of data collection, and then 3 spectra were collected of each individual plant in succession from Plant 1-Plant 5. Individual spectra represent 10 spectra, averaged internally on the FieldSpec Pro. The pistol grip was held approximately 10 -12 inches above the sugar beet canopy or in instances when the canopy was not sufficient, held in a manner in which only a representative leaf would be visible in the 28º field of view (FOV). The pistol grip was held between the data collector and the sun to minimize shadows in the FOV. A black shirt was worn at all times during data collection and the identification flag at each individual sugar beet plant was hidden from view with a black cover to minimize the likelihood of stray light contamination in an individual spectra. Digital photos of each collection site were taken either directly before or directly after spectra collection. Data from the Uof M plot sites and the NDSU potted plants was usually collected on the same day, while the field sites and the NDSU plot sites were usually collected on the same day if possible. Weather conditions during the summer, though, required 3 days of data collection each week to collect all of the data on several occasions.

The ASD data was then reformatted into Excel format and spreadsheets were developed that documented the data collected for each individual plant on each date of data collection. During the collection period, 2387 spectra of individual sugar beet plants and 804 digital photos were collected of sugar beets in various stages of Rhizomania infection. On August 25, upon completion of data collection, Plant 5 from each collection site and Plants 1A-1D at NDSU were collected, bagged, iced, and delivered to the NDSU Plant Pathology Lab for determination of Rhizomania infection by means of enzyme-linked immunosorbent assay (ELISA) analysis. The assumption was made that the health of Plant 5 was representative of each collection site and the health of Plants 1A - 1D was representative of the 20 NDSU plants.

DATA ANALYSIS

The 2387 spectra were organized into folders for Crookston, Glyndon, Fields, and NDSU. In each folder, files were developed for each collection date for each individual plant. The data for each collection date for each individual plant was then averaged and charted. The database now consisted of 6–7 weekly files for each of 115 individual plants. Each chart was visually inspected and any data that

appeared suspect was omitted from the database. 38 individual plant spectra were omitted in this manner. Suspect spectra could be the result of dirt contamination in the FOV or windy conditions at the time of spectra collection.
A chart interpolating various levels of dirt contamination the FOV was also developed as a visual aid to determine possible dirt contamination (Figure 2).

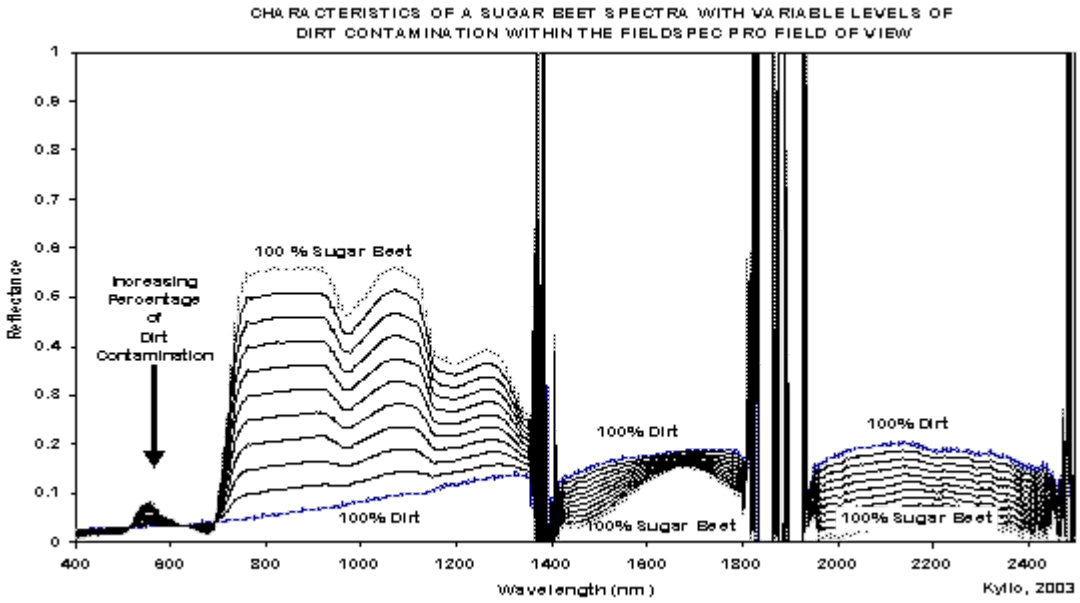

Figure 2. Spectra illustrating various levels of dirt contamination in a FieldSpec Pro FOV.

In the secondary step of data analysis, the spectra of all five plants collected on a specific date were aggregated, averaged, and charted. Data falling outside of the range of ± 1.3 standard deviations of the mean for each date were omitted from the database. The NDSU plants at this time were omitted from the database to ensure the integrity of the database. Comparisons of the spectra collected at NDSU and the spectra collected from plots and fields showed significant spectral differences that were attributable to stresses placed upon the sugar beets at NDSU from growth in pots. Omission of the NDSU data meant 513 spectra omitted from the database, but 1836 spectra still remained. The standard deviation analysis of the plot and field data then resulted in omission of 114 data files from the data base.

The database now consisted of representative spectra from each of 19 sites for each date of collection, which was either 6 or 7 collection dates, depending upon the site. The data was then classified into Rhizomania Positive, Rhizomania Negative, or Low Titer Positive, dependant upon the results of the ELISA tests. Results of the ELISA test showed 6 Rhizomania Positive sites, 7 Rhizomania Negative sites and 6 Low Titer Positive sites (Figure 3). A Low Titer Positive result indicates the possible presence of the Rhizomania virus but at a level too low for positive confirmation.

Rhizomania Positive	Rhizomania Negative	Low Titer Positive
Crookston Site 2	Crookston Site 1	Glyndon Site 3
Crookston Site 3	Field 1	Field 2
Glyndon Site 1	Field 3	Field 4
Glyndon Site 2	Field 5	Field 6
Field 7	Field 8	Field 10
Field 13	Field 9	Field 11
	Field 12	

Figure 3. 2003 research sites ELISA test results

Rhizomania Positive, Rhizomania Negative and Low Titer Positive folders were then developed with the results from each site classified into week of collection date, Week 1 (July 7–11), Week 2 (July 14–18), Week 3 (July 21-25), Week 4 (July 28-August 1),

Week 5 (August 4-8), Week 6 (August 11-15) and Week 7 (August 18-22). The data from each site, depending upon its disease status, was then aggregated, averaged and charted for each week. Again, data falling outside of ± 1.3 standard deviations of the mean was omitted from the database. The database now consists of a representative spectra for Rhizomania Positive, Rhizomania Negative and Low Titer Positive plants for each week of data collection.

PRELIMINARY RESULTS

It is quite early in this research project to make definite conclusions, but initial observations of the data indicate that there may not be a single spectral signature of Rhizomania infected sugar beets, but rather changing spectral characteristics indicating the presence of disease throughout a growing season. The spectra of a Rhizomania infected sugar beet with classic visual symptoms was compared with the healthy composite spectra of Week 7 and the spectra of Field 7, which was Rhizomania positive. On Week 6, a pattern begins to appear in the area of the spectrum centered at approximately 564 nm (Figure 4). The spectra of Field 7 begin to trend towards that of the spectra of a sugar beet with visible Rhizomania symptoms and away from the spectra of a healthy sugar beet. This may be significant for several reasons. This is the area of the spectrum in which nitrogen deficiency would be apparent on sugar beet spectra, but, the spectra of the healthy sugar beet is a composite of 35 sugar beet plants collected at 7 different collection sites during Week 7 and the likelihood of nitrogen deficiency in Field 7 and not the 7 collection sites that tested Rhizomania Negative during Week 7 is unlikely. The other significant feature of this comparison is the increase in the peak at 564 nm from Week 6 to Week 7 in Field 7, which in digital photographs taken on August 21, did not have visual indications of Rhizomania. If this pattern proves to be consistent throughout data analysis, it may be possible to determine not only areas of Rhizomania infection before visual symptoms appear, but it may also be possible to determine the stage of infection, both of which should be detectable with remote sensing.
Research (Steddom et al. 2003) suggests vegetative indices developed from this area of the spectrum as a means to quantify changes in pigment levels that may be indicative of Rhizomania infection and that is a possible means of early detection that will explored thoroughly in this project.

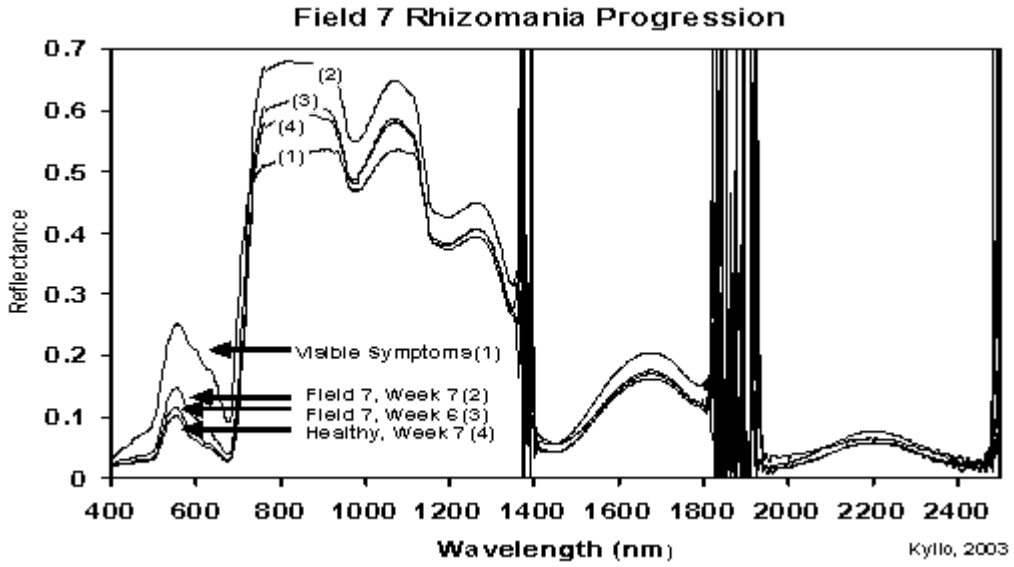

Figure 4. At approximately 564 nm, the spectra from Field 7 collected during Week 6 begins to deviate away from that of the healthy composite spectra collected during Week 7 towards the spectra of the visually symptomatic sugar beet. Deviation increases for Field 7 data collected during Week 7.

2004 RESEARCH GOALS

There are several distinct objectives for research that will be conducted on this project during the 2004 growing season.

Objective 1. Definition of the spectral characteristics of Rhizomania infected sugar beets.
Objective 2. Determination of WHEN spectral characteristics of Rhizomania infected sugar beets. become detectable.
Objective 3. Development of a method to detect the spectral characteristics of Rhizomania Utilizing remote sensing methods.
Objective 4. Expansion of the database.

CONCLUSION

Although early in the analysis of data collected during the 2003 sugar beet growing season, it appears as though several patterns are beginning to emerge. There appears to be no specific spectral signature of Rhizomania infected sugar beets, but there appear to be spectral *characteristics* in the range of 400-800 nm that may indicate disease detection and they will be explored in detail during the second year of research. It also appears that it may be possible to detect Rhizomania infection before it is detectable by visual means from the ground, if initial observations prove to be correct. It should be kept in mind that any patterns appearing to emerge thus far are only preliminary observations. It will be vitally important to the success of this project to further develop a large, well organized database.

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REFERENCES:

Steddom, K., Heidel, G., Jones, D., and Rush, C. M. 2003. Remote detection of rhizomania in sugar beets. Phytopathology 93:720-726.