- Crosses of Scott's lines (*L. chilense*) with susceptible lines:

From the 74 plants of Marina x GS9, 18 plants had moderate to slight-moderate symptoms. Fruit were collected from four plants.

2.1.4. Observations from the Salama Valley

The Salama Valley is about 4 hours by car from Guatemala City (NE of the City), and it is a major tomato growing area. They have had serious problems with begomoviruses and more recently with a problem called chocolate spot (unknown causal agent). AgroVida (a Bayer Co. and GTZ sponsored IPM program in Salama Valley) included Llanero 7 (a GF line x susceptible line) in their field evaluation. It was highly resistance to the begomoviruses and the chocolate spot. Currently, 20 g of this hybrid was prepared in Israel and has been sent to Guatemala for field testing in grower's fields.

We observed a new planting of Elios that had 100% chocolate spot and another large field with 35-day-old transplants of Silverado, which had 100% incidence of begomoviruses. Unfortunately, for this latter case, other tomato fields are being planted adjacent to this field. These two fields provide excellent opportunities for studies on the economic impact of these diseases. Follow up from March 2003, a large field (2.8 ha) with 35-day-old transplants of Silverado, which had 100% incidence of begomoviruses, was observed in March. The yield from this field was 100 boxes of 50 lb of tomatoes. The break even point would have been 2,800 boxes and the expected yield was 8,000 boxes. Price of tomatoes varies, but about $10/box could be used. Thus, there was a $28,000 investment and a return of $1,000. Expected return was $80,000. Again this September 2003, the tomato fields in this area had a high incidence of geminivirus infection.

2.1.5. Family garden/school garden project:

In the area around San Miguel Chicaj (west of Salama City), there are many small Mayan growers. They are plagued by begomoviruses in their peppers and tomatoes. In cooperation with World Vision and AgroVida, we will attempt to distribute a tomato hybrid for home gardens (huerto familiar / huerto escolar). This hybrid will have high resistance to begomoviruses and excellent yield for small, flavorful fruits. The idea is that this can be planted for home consumption; and thus, there is not the need for a firm fruit with the roma-type shape. Pilones de Antigua Company is producing this hybrid seed.

2.1.6. Other tomato problems:

Chocolate spot — plants with typical chocolate spot symptoms were collected and tested for *Tobacco streak virus*, *Tomato spotted wilt virus*, and potyviruses. One sample gave a positive potyvirus reaction.

Fruit necrosis – this problem was evident is some cultivars at the Barcena Technical School, where there is a large field trial. Several samples were tested for *Tomato bushy stunt virus* and all were negative.

Wilting syndrome – this is a problem in a valley near Agua Blanca and has reduced production for 560 ha to about 105 ha. It appears to be caused by a soil-borne pathogen, which was identified in May 03 by Dr. C. Allen as *Ralstonia solanacearum*, which causes bacterial wilt. Tomatoes produced here are shipped to Salvador and are mainly the hybrid Sheriff.

These three problems need serious attention as they are impacting tomato production and no controls are known.

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2.2. Evaluation and selection of germplasm in Guatemala – September 2003

2.2.1. Field test

Two hundred and eight populations (7,600 plants) were evaluated at 90 days after transplanting by the research team, and all had mature fruit. The field test was severe and the commercial cultivar, Marina, had a DSI of 3 (4 most severe rating). During the dry season, Marina has a DSI of 3.5 to 4. A commercial planting of Marina, which was about 400 meters away from our field plot, was a total loss, and the grower did not harvest any fruit.

Shape, size, and firmness were major criteria for selection of the plants for future testing. A firm fruit with a saladette shape (roma shape) of about 120 g is the most desirable fruit type for Guatemala. Silverado, Marina, Toro, and Elios are popular tomato cultivars for the major markets in Guatemala. Round tomatoes are available in the "up scale" grocery stores and the Sheriff-type is sold to San Salvador.

2.2.2. Conclusions

Most resistant hybrid: The F1 hybrid (175) from GS16 (*L. chilense* resistance) x GF13 (*L. hirsutum* resistance) was the most resistant and DSI was 0.5 -- no symptoms were evident. Fruit is round and medium firm at about 120 g.

Progress on fruit size and shape:

- Large round (>150 g): Number of plants selected for F2, F3, F4, and F6 populations were 6, 3, 5, and 1, respectively.
- Medium round (ca. 120 g): Number of plants selected for F2, F3, and F4 populations were 16, 11, and 3.
- Blocky types (ca. 80-120 g): Number of plants selected for F2, F3, and F4 populations were 6, 4, and 11.
- Saladette types (120 g): Number of plants selected for F2, F3, F4, F5, and F6 populations were 2, 6, 6, 1, and 2.
- "Cucumber" shape (ie, long and slender): Three F3 plants were selected.
- Cherry type: Number plants selected for F2 and F3 populations were 1 and 1.

2.2.3. Other tomato disorders:

- Gray Leaf Spot: The populations with TY197 or TY198 in their background had indeterminate growth and relative low yields and showed susceptibility to gray leaf spot. The parental plants (F2) were selected because of their vigor and this was during the dry season. Also, some of these parental lines (F2) had excellent flavor. Selections of resistant and susceptible plants for gray leaf spot were made to use in our marker-assisted selection studies.
- Bacterial wilt: Over the past three growing seasons, several plants had shown severe wilting and had died. This time it was more serious than in the past, and about 10% of the plants were lost. Jay Scott, University of Florida, identified the problem as bacterial wilt. The next planting will be in a different field, which does not have a history of this bacterial wilt problem. Since the project must rent land, it is not always possible to rent the best field for growing tomatoes.
- Fruit disorder: Jay Scott pointed out several disorders on the fruit, eg golden fleck, zipper markers, cat claws, and whitefly injury.

Selection for parental lines for hybrid production:

F4, F5, and F6 plants will be used in crosses to create hybrids of potential interest. These hybrids will include the saladette types, the Sheriff-types, and the large round types. Currently, the plan is to have the crosses made at Pilones de Antigua and this company will give the seed to our program, plus assisted in the field evaluation of these crosses.

Crosses of Scott's lines (*L. chilense*) with susceptible lines has led to a new line called, GS173-1 (a new line planted in Guatemala for the first time), has the saladette shape and excellent resistance. It will be used in crosses with lines with *L. hirsutum* resistance to create several hybrids.

2.3. Generation of DNA markers linked to resistance to geminiviruses

PCR fragments corresponding to probes TG297 and TG436, located on tomato chromosome 6, obtained from DNA of different resistant lines were sequenced. The nucleotide sequences corresponded to *L. esculentum*, *L. chilense* and *L. hirsutum*. This work was conducted during a training visit in the laboratory of Dr. Douglas P. Maxwell and Dr. Mike Havey in Madison Wisconsin. This collaborative effort at the University of Wisconsin-Madison was conducted by Dr. Luis Mejia and Ing. Amilcar Sanchez from College of Agronomy of Universidad de San Carlos in July 2002 and July 2003, and by Andreas Pallides, a student of Dr. Hanokh Czosnek from the Hebrew University of Jerusalem, Rehovot, in July 2002.

SNP and indel analyses of F2 populations from crosses of breeding lines (GF1, GF2) from the FAVI hybrids by M82 or HC7880 did not provided support for the introgressions from *L. hirsutum* to the *TY 1* gene (*L. chilense*) region in upper arm of chromosome 6 or the lower arm of chromosome 11, which has the introgression from *L. hirsutum* in line H24.

A search for an introgression of *L. hirsutum* in Chromosome 6 in the region of the *Ty1* gene from *L. chilense* of tomato genotypes resistant and susceptible to begomoviruses was conducted in July 2002. Lines tested were: TY52 line (homozygous for the resistance gene, *TY1*) and TY50, an isoline of TY52, but susceptible; GF lines from Guatemala, the wild species, *L. hirsutum* (DNA supplied by D. Spooner, UW-Madison), M82 and HC7880 lines, susceptible in Guatemala. Initially, several RFPL markers for the *Ty1* gene-region in Chromosome 6 were sequenced, and PCR primers designed from the TG436 RFLP marker were used to amplify fragments and these were cloned and sequenced. Two indels, each with two nt insertion/deletion, were detected among the lines. TY52 and GF1 had the identical sequence, and the other lines had the identical sequences, thus the lines were divided into two groups. This 2002 summer experience provided an introduction into this technology and indicated that F2 populations should be analyzed for segregation of these two indels.

Additional research/training was conducted in July 2003 by Mejia and Sanchez for introgression of *L. chilense* and *L. hirsutum* in Chromosomes 6 and 11. A new technique was used in which PCR fragments were labeled with a fluorescent dye and the fragment length detected on an ABI sequencer to detect the indels. These studies were conducted with plant material that has been planted in Sanarate valley, Guatemala, and selected for resistance/susceptibility to geminivirus-diseases. Additional germplasm was included which originated from breeding programs from Dr J. Scott (U. Florida) and Dr. Peter Hanson (AVRDC, Taiwan).

The following germplasm was used for these studies:

- GS9: a line selected in Guatemala from a Jay Scott's *L. chilense* line. This line showed excellent resistance in Guatemala and is used as a breeding line in our program.

- GF2, GF13: lines selected from Czosnek/Vidavski's *L. hirsutum* line: they showed excellent resistance in Guatemala and are used as a breeding line in our program.
- HC7880, a very susceptible open pollinated line from Cuba, which has excellent yield and heat tolerance.
- M82, a very susceptible line from Israel used in mapping studies by Dr. Zamir.
- *L. esculentum* var. *cerasiforme*, a wild-type tomato from Guatemala, which was collected in Sanarate, GT.
- Better Boy, a hybrid from Seminis Seeds Co. It is heterozygous for the Mi gene marker (REXF1 and REXR2) from sequence data, and is used as a tool to verify our marker system.
- CLN2443A, a line developed by P. Hanson, AVDRC; it is derived from line H24 that has resistance, *Ty2* gene, to TYLCV from *L. hirsutum* f. *glabratum*.
- *L. hirsutum* LA 1928 and LA 1223 was used as wild tomato species; it was obtained from Dr. D. Spooner (U. Wisconsin).
- F2 populations form crosses of GF lines with either HC7880 or M82, where resistant and susceptible plants were selected in the field.

DNA from the parental lines and from the selections was used to screen for the indel marker in region 436 of Chromosome 6 and additional markers corresponding to chromosome 11. The selection of primers was based on published information for the location of the genes for resistance. Sequences for ends of some probes were obtained from the Cornell Web site (www.sgn.cornell.edu), however most probes were sequenced.

For the TG436 marker region, the two indels detected by sequence data and fluorescent-dye tagged PCR fragments segregated randomly in the four F2 populations evaluated, which indicates that this region, which is near the *Ty1* gene, does not have the introgression form *L. hirsutum*, origin of GF1 and GF2.

For the Chromosome 11 region corresponding to the introgression for *Ty2* gene between TG39 and TG393, PCR primers were developed for seven markers in this region. Of the PCR derived fragments, those associated TG30, TG105, TG26, T1660, CT120A, and TG393 were sequenced from the various lines. No polymorphisms were detected in the sequence of the fragments for the marker regions, TG30, TG26, TG393, for the susceptible GF tomato lines and susceptible lines. For the TG105 PCR fragments, GF2 had many polymorphisms, which separated it from the other lines. In an analysis of the two F2 populations, there was no correlation between viral resistance and the segregation of this marker region.

Since this region was shown to have an introgression for the *Ty2* gene from *L. hirsutum* f. *glabratum*, the value of this PCR/sequence approach was tested with line H24 from AVDRC, which has this introgression. Sequences for the TG105 fragment for H24 and two *L. hirsutum* accessions had identical sequences, which were different than the sequences for all other lines tested. This sequence differences were 9 SNPs and a large 13-nt indel, all within 270 nt region, which clearly demonstrated the power of this approach for detection of introgressions from wild species.

The results from this approach indicated that the markers could be used to follow segregation in F2 populations, to tag chromosomal fragments from wild tomato species introgressed into the domesticated tomato, and to distinguish between homozygous and heterozygous regions.

Another search was conducted in the H. Czosnek lab at the Hebrew U. using similar approaches. Markers shown to be included, or excluded, from chromosomal introgressions from the wild tomato species (chromosome 6 from *L. chilense* and chromosome 11 from *L. hirsutum*) were used. PCR primers based on the sequence of these markers were designed and used to amplify chromosomal fragments, which were subsequently sequenced and compared for polymorphism possibly related to resistance/susceptibility to begomoviruses.

- Chromosome 6: TG297 and TG436 (in the *L. chilense* introgression).
- Chromosome 11: TG36, TG26, TG393 (in the *L. hirsutum* introgression), and TG286 and TG546 (outside the introgression).

The markers were used to amplify DNA fragments from the following germplasm:

- The wild species *L. hirsutum* (LA 1223, LA 1353, LA 1928 and LA 386).
- Isoallelic lines tolerant and susceptible to TYLCV, with and without gene Ty-1 originating from the *L. chilense* (TY 52 and TY 50).
- Lines selected for resistance to geminiviruses in Guatemala (GF-1, and GF-3 from hybrid FAVI-9, and GF-2 from FAVI-12) with resistance originating from *L. hirsutum*.
- Susceptible *L. esculentum* line HC-7880.

The PCR-amplified fragments were sequenced and compared: no differences related to begomovirus resistance for the GF lines and susceptibility was observed. There was a difference between TY52 and TY50 for the TG436 marker.

2.4. Evaluation of DNA markers linked to resistance to root knot nematode, the Mi gene

The Mi gene was proposed as the marker to be used to introduce this technology into the curriculum/training at San Carlos University. A graduate student at San Carlos University, Carolina Zeae, used PCR and restriction digestions, to follow the REX PCR fragment, which is associated with the *Mi* gene in F2 populations [Marina (*Mi* gene) x H5 [hybrid with *L. hirsutum* resistance (GF) and *L. peruvianum* resistance (GP10, TY198 source)] from plants selected in the field in March 2003. Her results were compared with sequence analysis of the REX PCR fragment from F3 seeds from F2 plants selected in the field. Better Boy was found to be heterozygous for the REX region, and all three classes of genotypes were detected by both PCR and restriction digestions, and sequence of PCR fragments. Ms. Zeae's thesis is being completed in the fall of 2003.

2.5. Web site

Details and photographs of field plots can be found on the web site prepared by Dr. Douglas P. Maxwell: http://www.plantpath.wisc.edu/GeminivirusResistantTomatoes CDR

3. Plans for next year

3.1. Seedling production and hybrid seed production

Two of the three large tomato seedling producing companies, Pilones de Antigua and Agropecuana Popoyan, in Guatemala were contacted and managers of both companies indicated that they are interested in producing the seedlings and helping us locate growers for

field testing. Also, Pilones de Antigua is producing hybrid seeds. The general manager, Richard Rotter, was the general manager for Asgrow Seed Co. in Salama and has considerable experience in hybrid tomato seed production. In July and September, intellectual property discussions were initiated for the resistant lines developed in Guatemala. Also, discussions have been initiated to start a private seed company as sub-division of Pilones de Antigua and this company would produce tomato hybrids for Central America and the Caribbean Region from germplasm generated by the program at San Carlos University.

3.2. Field evaluation of additional populations

A large field planting (6,000 plants) is planned for December 2003 and evaluation for March 2004. F2, F3, F4, F5, and F6 populations will be included to stabilize resistance and horticultural characteristics. F1 populations from crosses with resistance from either *L. chilense* or *L. hirsutum* will be planted and evaluated for the viruses that are infecting plants with these different sources of resistance genes.

3.3. Economic threshold for geminivirus in tomato

Growers need a predictive method to determine if it is worthwhile to continue to manage a tomato field. The development of an economic threshold, which would relate percentage of plants with various DSI at 45 or 60 days with yields, would be one approach.

The technical representatives from AgroVida, Salama, Guatemala are in the process of collect data to prepare information for an economic threshold that will take account the percentage of plants infected with geminiviruses and predicted yields.

3.4. DNA markers linked to resistance to begomoviruses

Our main approach in the proposed research was to search for polymorphic markers defining the chromosomal introgression(s) from the wild species into the domesticated tomato, which confer resistance to begomoviruses, in the GF lines from Guatemala. SNPs and indels have been used to show that the resistance in GF lines is not associated with the same introgression of *Ty1* and *Ty2* genes in Chromosome 6 and 11, respectively. This approach will next be tested with the resistant lines selected in Guatemala from TY197 or TY198, GL11, and from J. Scott lines, GS9.

Additionally, an initial RFLP approach will be used with at least two markers for each of the 12 chromosomes to evaluate other regions for polymorphisms. Along with this, it might be possible to initiate an AFLP approach at University of Wisconsin-Madison in summer 2004. At least three scientists from Guatemala are expected to participate in this training in summer 2004.

3.5. Workshop

A workshop on molecular methods for the detection of geminiviruses and *Ralstonia* and marker assisted selection is being organized for February 25-27, 2004. This workshop will take place at the Faculty of Agriculture, San Carlos University. The support of the Guatemalan Council of Science and Technology (CONCYT) has been requested to provide matching funds. It is expected that around 20 participants from Guatemala will be trained in these molecular techniques.

4. Managerial issues

The Volcani Center and the Hebrew University of Jerusalem received advances to cover expenses. Problems related to fund transfer to the University of San Carlos that arose at the beginning of the research have been much improved.

5. Special concerns

During the last field assays, summarized in September 2003, it appears that problems caused by Bacterial wilt were responsible for damages unrelated to the presence of whitefly-transmitted geminiviruses. We will need to deal with this problem, which may develop considerably, in the near future.

6. Strengthening

6.1. Equipment

Equipment and materials were obtained while at the University of Wisconsin in July, 2002. These include a Thermal Cycler (MJ Research, PTE 200), a UV cross linker oven, micropipettors and other miscellaneous supplies and reagents. All these supplies were brought back to Guatemala by Luis Mejia and Amilcar Sanchez. The laboratory at the College of Agronomy of Universidad de San Carlos is currently fully operational in the detection of geminiviral DNA in plant tissue. These items, and others that were used during the work that was conducted in Madison, were paid by the funds which were transferred to the University of Wisconsin. A Dell Inspiron 8200 laptop computer was obtained for the Guatemalan partners, which was paid from the budget assigned under Materials in the Guatemalan budget. A gel documentation system was purchased in Madison in summer 2003 and carried to Guatemala.

6.2. Training in the use of DNA markers

Dr Luis Mejia and Ing Amilcar Sanchez have spent two research visits in the laboratory of Dr Douglas Maxwell and Dr. Mike Havey, July 2002 and July 2003. They have learned to several technologies associated with Marker-Assisted Selection: PCR primer design from RLPF marker sequences, cloning and sequencing, BLAST searches, and sequence comparisons, and fluorescent-dye tagged PCR fragment analysis. DNA extractions and PCR analyses have been integrated into the technology at San Carlos University.

7. Collaborative activities

6.1. Visits and field evaluations

1. Dr. Favi Vidavski, Plant Breeder from the Hebrew University of Jerusalem, visited the project in Guatemala in January, 2002. A breeding program to build tomato lines with resistance to whitefly transmitted geminiviruses and other

economically important pathogens, which could be combined into commercial hybrids for the Guatemalan market, was discussed and decided upon.

2. Drs Douglas P. Maxwell, Favi Vidavski, Henryk Czosnek, Jay Scott (from the University of Florida), together with Dr Luis Mejia, visited the field plots in January 2003. Dr. Maxwell returned for final evaluation of the test in March 2003.
3. Drs Doug Maxwell and Jay Scott, together with Dr Luis Mejia, visited the field plots in September 2003 for final evaluation of this third field test. Dr. Maxwell reported results of the March and September 2003 field tests in the project web site.

6.2. Production of advanced breeding lines

About 20 g of seeds of Llanero 7 (GF1 x commercial breeding line) have been produced by a commercial company in Israel at 1.5 cents/seed. The seeds have been sent mid-October 2003 to Dr Mejia, Guatemala. Four other hybrids, Llanero 10, Llanero 11, Llanero 12 and Maya Garden are being produced at Pilones de Antigua. These hybrids were produced from crosses between geminivirus resistant lines that have been selected in Guatemala. All five hybrids will be field tested at different locations in Guatemala in early 2004.

6.3. Annual meeting and Workshop

The first annual meeting of the CDR project has taken place in Guatemala from January 19 to January 27, 2003, in Antigua, Guatemala. Drs. Douglas P. Maxwell and Michael J. Havey from the University of Wisconsin and Drs. Hanokh Czosnek and Favi Vidavsky from the Hebrew University of Jerusalem have attended this meeting, together with Dr. Jay Scott from the University of Florida.

A symposium of breeding for resistance to whitefly transmitted geminiviruses in vegetables took place on January 23, the Council for Science and Technology of Guatemala (CONCYT) provided funds for this event along with the CDR/USAID grant. Approximately 80 local scientists, breeders, extension persons, and representative of the tomato industry have participated. The following lectures have been given:

1. Douglas P Maxwell (U. Wisconsin, USA): Geminiviruses in Central America and the Caribbean.
2. Henryk Czosnek (Hebrew U., Israel): Whitefly-virus interaction.
3. Luis F. Orellana (Bayer crops Science, Guatemala): Chocolate spot: a new disease of tomatoes in Guatemala.
4. Hector Ramazzini (Pegon Piloncito, SA, Guatemala): The market of tomato seed in Guatemala.
5. Michael J. Havey (U. Wisconsin, USA): Molecular marker assisted breeding.
6. Favi Vidavski (Hebrew U., Israel): Introgression of resistance to geminiviruses from wild species.
7. Luis Mejia (San Carlos U., Guatemala): Breeding for resistance to geminiviruses in Guatemala.
8. John W. Scott (U. Florida, USA): Breeding and genetics of geminivirus resistance in tomato.
9. Mayte Piñón (Instituto de Investigaciones Horticolas Liliana Dimitrova, Cuba): A strategy for breeding tomatoes for resistance to begomoviruses

This meeting also provided an opportunity for interaction with a Cuban scientist who is also breeding tomatoes for resistance to begomoviruses.

Drs. Vidavski and Scott, the two plant breeders, organized a workshop on methods for field evaluation of tomatoes for horticultural characteristics and a scoring system was developed, which was used in March and September. This system will be introduced into the Tomato-MERC group at the field evaluation in Morocco in December 2004.

6.4. Linkage with Middle East Regional Cooperation (MERC) Program

Dr Luis Mejia has attended the annual meeting of the MERC program on classical and marker assisted breeding for TYLCV resistance, and the workshop on classical and molecular-assisted breeding, which were held in Malaga, Spain, February 7-14, 2003. Dr Mejia has presented the ongoing breeding work in Guatemala and how the germplasm developed during the MERC and CDR projects could complement. He also participated in the first field evaluation of tomato germplasm in Morocco in February 2003.

Dr. Mejia and his colleagues have made crosses of local varieties from Tomato-MERC grant countries (Lebanon, Egypt, and Jordan) with GF lines and GS lines and sent these F1 seeds to these countries.

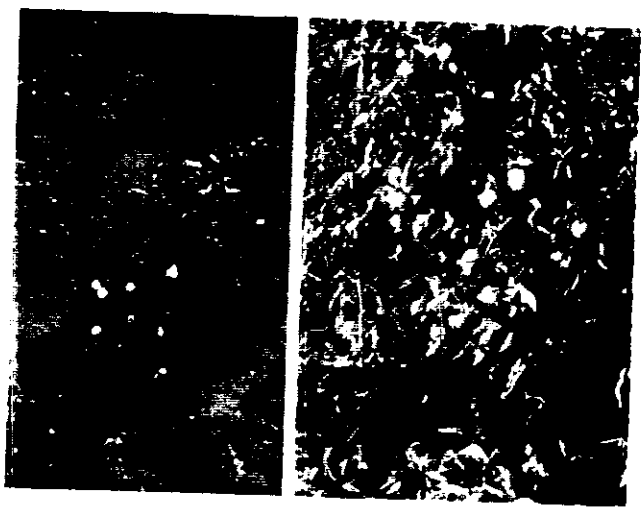
Five tomato lines from Jordan and four tomato lines from Lebanon were sent to Dr. Luis Mejia in Guatemala. These lines were crossed to GF13 and GS16, the most resistant lines selected in Guatemala. The hybrid seeds were recently sent to Drs. G. Anfoka in Jordan and Dr Y. Abu-Jawda in Lebanon.

7. List of papers and abstracts

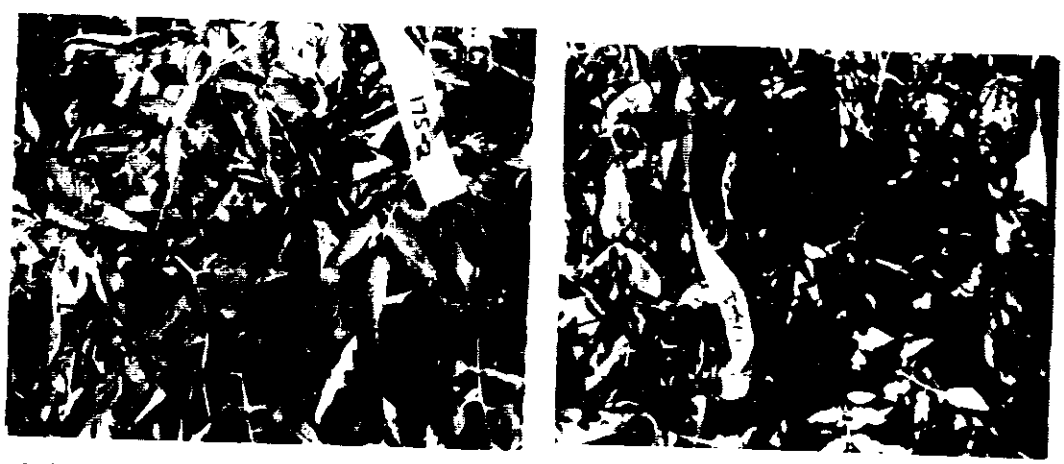
L. Mejia, R.E. Teni, H. Czosnek, F. Vidavski, A. Bettilyon, M.K. Nakhla and D.P. Maxwell. 2002. Field evaluation of tomato experimental lines and hybrids for resistance to begomoviruses in Guatemala. *Phytopathology* 92:S54.

Mejía, L., R. E. Teni, A. Sánchez, A. Pallides, F. Vidavski, H. Czosnek, L. Cohen, M. Lapidot, J. W. Scott, M. J. Havey, M. K. Nakhla and D. P. Maxwell. 2004. Evaluation of tomato germplasm and selection of breeding lines for resistance to begomoviruses in Guatemala. To be presented at the 4th International Geminivirus Symposium, Capetown, South Africa, February 16-24, 2004.

Total loss of tomato (January 2003)



Left: Elios, commercial cultivar. Right: H31: F2 plant with resistance from *L. Hirsutum* and *L. peruvianum*, 85 days after transplanting (January 2003).



F1 hybrid 175 from GS16 (*L. chilense* resistance) x GF13 (*L. hirsutum* resistance). Most resistant hybrid with no symptoms (left). Fruit 120g is round and medium firm (right) (September 2003).