

# Other non-chemical control methods

Session V



## Host resistance to whitefly-transmitted geminiviruses

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During the last two decades, there has been a worldwide spread of the B biotype of *Bemisia tabaci*. The severe out-breaks of the B biotype in tropical and subtropical regions was accompanied by the emergence of whitefly-transmitted geminiviruses. Geminiviruses comprise a large and diverse family of viruses that infect a wide range of important crop plants and cause significant yield losses. The family *Geminiviridae* is divided into four genera, one of which is *Begomovirus*. Species of this genus infect dicotyledonous plants and are transmitted by the whitefly *B. tabaci* (Gennadius) in a persistent, circulative manner. Control measures in infected regions have traditionally emphasized vector control, mainly by pesticides or physical barriers. Chemical control methods have been only partially effective, since the vector has to be eliminated before it transmits the virus. Furthermore, there are concerns that the vector may develop pesticide resistance and the intense application of pesticides may have deleterious effects on the environment. Physical barriers such as fine-mesh screens and UV-absorbing plastic sheets and screens have been shown to inhibit penetration of whiteflies into greenhouses. Nonetheless, adoption of physical barriers adds to production costs and these screens create problems of shading, overheating and poor ventilation. However, under conditions of severe whitefly attack, none of these control measures has sufficed to prevent virus spread. Thus, the best way to reduce geminivirus damage is by breeding crops resistant or tolerant to the virus, either by classical breeding or by genetic engineering.

## **Cultural practices for managing *Bemisia tabaci* and vectored viral diseases**

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Cultural controls may be defined as methods of manipulating pest populations through agronomic or horticultural practices. Actually, they constitute the set of pest management tactics not considered as chemical or biological. Like host plant resistance, they are usually preventative in seeking to create unfavorable conditions for the development of pest populations. Vectored viruses are an additional factor in whitefly management that predisposes certain strategies over others. Exclusion with screen netting or crop-free periods are two alternative tactics that have proved effective and often indispensable but are expensive in the first case or require area wide compliance in the second. Living and reflective mulches have shown clear benefits but are not yet widely adapted due to cost, unavailability of materials or incompatibility with standard practices. Trap crops, UV screens and reduced fertilization seem to work in some situations although determining factors are not clear and adaptation is therefore limited. Windbreaks, mass trapping and rogueing are easily adapted even in the absence of convincing evidence because they are intuitive and/or are easy to implement. It is unlikely that any whitefly management system could be successful without including one or more cultural controls, given the wide range of tactics included under this rubric.

## **Host plant resistance to whitefly transmitted Cotton leaf crumple disease**

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Cotton, *Gossypium hirsutum* L., cultivars and breeding lines were evaluated in the field for resistance to the Cotton leaf crumple (CLCr) disease caused by Cotton leaf crumple virus (CLCrV) (CLCrV, Genus Begomovirus, Family Geminiviridae) transmitted by silverleaf whitefly, *Bemisia argentifolii* Bellows and Perring. Cultivars and breeding lines were evaluated in field plots during 1999 through 2001 in the Imperial Valley, CA. Cotton entries were rated for severity of CLCr disease symptoms, and the presence of CLCrV in leaves of selected plants of each cultivar/breeding-line was determined by dot blot hybridization with a CLCrV DNA probe and/or PCR analysis with degenerate geminivirus primers. DNA sequencing of geminivirus DNA-A and DNA-B fragments, PCR-amplified from symptomatic cotton plants, confirmed geminivirus infection and allowed for partial characterization of CLCrV. Differences in whitefly infestation levels and virus disease severity were detected among cotton entries. The breeding line NX 2387C, with Cedix parentage, had the lowest CLCr disease severity ratings and level of CLCrV infection, suggesting a high level of virus resistance. Lines DG 2165 and DG 2383 had few or no CLCr symptoms and lower levels of virus infection, whereas Stoneville 474 and NX 2207 had visible CLCr symptoms and higher levels of virus infection. The cultivars AP 4103 and AP 6101 had the highest CLCr disease ratings and high levels of CLCrV, based on squash blot, dot blot and PCR analyses

## **Search for genetic components of resistance to *Bemisia argentifolii* oviposition in tomato using *Lycopersicon pennellii* accession LA716 introgression lines**

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Methodology for identifying individual genetic components of resistance to the silverleaf whitefly, *Bemisia argentifolii*, in controlled environment studies was developed and validated through comparison to results of a previous study using six commercial tomato, *Lycopersicon esculentum*, lines. The entire genome of the wild tomato species *Lycopersicon pennellii* LA 716 was surveyed for resistance to *B. argentifolii* by determining choice and no choice oviposition and nymphal development on fifty introgression lines compared to the *L. esculentum* parent variety M82 and another commercial variety E6203. Twenty-one of the lines had mean ( $\pm$  SE) eggs per terminal leaflet ranked below that of M82 ( $0.19 \pm 0.05$ ), ranging from  $0.00 \pm 0.00$  to  $0.17 \pm 0.12$  in the choice study. Eleven of the lines had significantly ( $P < 0.05$ ) fewer eggs per leaflet than did E6203 ( $0.42 \pm 0.07$ ). Choice oviposition in three subsequent studies of eleven selected lines resulted in four lines having significantly ( $P < 0.05$ ) fewer eggs per leaflet than did either commercial variety. Twelve of the lines had significantly ( $P < 0.05$ ) fewer mean ( $\pm$  SE) eggs per terminal leaflet than did M82 ( $13.77 \pm 0.84$ ) ranging from  $2.00 \pm 0.55$  to  $8.75 \pm 0.53$  in the no choice study. Twenty-two of the lines had significantly ( $P < 0.05$ ) fewer eggs per leaflet than did E6203 ( $16.52 \pm 1.07$ ). No choice oviposition in a subsequent study of eight selected lines resulted in six lines having significantly ( $P < 0.05$ ) fewer eggs per leaflet than either commercial variety. Selected lines were further tested as homozygotes and heterozygotes for the *L. pennellii* introgression fragment.

## **Characterization of host plant resistance in alfalfa against *Bemisia***

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Thirty-eight plants were taken from a University of California alfalfa selection nursery for developing resistance to silverleaf whitefly, *Bemisia argentifolii* Bellows & Perring. Seventeen of the plants had low whitefly infestation and were categorized as 'potentially resistant;' 21 of the plants had high whitefly infestation and were categorized as 'presumed susceptible.' Plants were propagated vegetatively so that replicated measurements of whitefly performance could be made on each genotype. Two colonies of silverleaf whiteflies were used: one reared on alfalfa (alfalfa-experienced whiteflies), and the other on cotton (alfalfa-naive whiteflies). The effect of variation among alfalfa genotypes on whitefly performance was similar for both whitefly sources, although on all genotypes, the alfalfa-experienced whiteflies generally performed better than their alfalfa-naive counterparts. In greenhouse tests, fecundity of newly eclosed adults (over a 5 day period) on the 17 potentially resistant genotypes was relatively consistent in being lower than fecundity on the presumed susceptible genotypes. However, in nymphal survival tests, the response on the 17 potentially resistant genotypes was not consistent. Nymphal survival (egg to adult) on some of these was very low, as expected, while nymphal survival on others was as high as on the presumed susceptible genotypes. Fecundity and nymphal survival data were not correlated for alfalfa-naive whiteflies, and were only weakly correlated ( $r^2 = 0.13$ ,  $df = 32$ ,  $P = 0.04$ ) for alfalfa-experienced whiteflies. Thirteen genotypes then were examined in the greenhouse in stage-specific survival tests, where four genotypes demonstrated high resistance (< 10% nymphal survival) and three demonstrated moderate resistance (11 - 34% survival) compared to the three presumed susceptible genotypes that were tested (51 - 73% survival). Most of the mortality on the resistant genotypes occurred in the first instar, while mortality was more evenly distributed across the life stages on the susceptible genotypes. Interestingly, on the resistant genotypes, if nymphs survived to second instar, then their subsequent survival to adult eclosion was similar to survival of second instar through adult on susceptible genotypes. Six of the genotypes used in the greenhouse stage-specific survival test also were evaluated in the field for nymphal survival, and these results were consistent with the greenhouse tests.

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## **Uv-blocking plastic covers and induction of systemic resistance in plants to control *Bemisia tabaci* and geminivirus infections in tomato in commercial plastic houses of southern Spain**

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The yellow leaf curl disease of tomato (TYLCD) is the main limiting factor for the production of tomato in the south of Spain. The causing agents are a complex of virus species of the genus Begomovirus (family Geminiviridae) that in nature are transmitted by the whitefly *Bemisia tabaci*. Control of TYLCD in protected tomato crops of southern Spain is difficult during the warm season (June to November) because of the high *B. tabaci* and therefore disease pressure present. Partial resistance to virus infection is available in a number of commercial cultivars that do not totally satisfy agronomic/commercial standards and insecticide treatments are not effective to control insect populations at levels in which TYLCD spread is controlled. Therefore we have investigated crop management strategies that could help to reduce damage caused by TYLCD. Between 1998 and 2002 several field trials were performed in open field and plastic house tomato crops of Málaga (southern Spain). Induction of systemic resistance in tomato plants using an analogous of salicylic acid (acibenzolar-S-methyl, Bion, Syngenta) resulted in increased tomato production in open field grown crops. In protected crops, we used photosensitive plastic covers blocking ultraviolet light (UV) to interfere with *B. tabaci* vision. This resulted in a significant reduction of *B. tabaci* population numbers and of TYLCD incidence in the not highly technified plastic houses typical of southern Spain ("parral" type) in which large lateral windows are completely opened most of the time. Consequently, increased tomato fruit yields were obtained. Combination of both systemic resistance induction and the use of UV-blocking plastic covers resulted in a significant fruit yield increase in protected tomato crops compared to either treatment used individually and to traditional crop management. Therefore integration of both strategies is a good alternative to reduce TYLCD damage in protected tomato crops of southern Spain.

## **Advances in physical control of TYLCV and its vector *Bemisia tabaci* in Sicily by UV-absorbing films**

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Among physical control methods of *Bemisia tabaci* and related virus problems, the use of photoselective plastics as covering material of greenhouses is increasing all over the Mediterranean basin. Filtration of the light spectrum in the UV band has shown to negatively affect the ecological behaviour of insect vectors, especially as regards orientation in flying, feeding and interacting between sexes, thus leading to a general diminution of virus spread on crops. Field experiments for evaluating the efficacy of photoselective covering materials have been started in Sicily from a couple of years. Preliminary comparative observations have already shown a reduced occurrence of both *B. tabaci* and TYLCV in greenhouses covered with UV-absorbing films. These encouraging results created a great interest among Sicilian tomato growers as well as local producers of polyethylene materials. Therefore, research on this topic is continuing in south-eastern Sicily, where the efficacy of locally produced films is presently under evaluation. Studies reported here have been carried out in three tomato greenhouses respectively covered with: (i) a conventional polyethylene film; (ii) a foreign produced UV plastic film; (iii) an UV-absorbing film made by a specialised local factory. Data were periodically recorded on levels of infestation by *B. tabaci* and the incidence of TYLCV infections. Both photoselective films under test gave excellent results, and reduction in TYLCV incidence has been statistically significant compared to the conventionally covered greenhouse, where higher densities of the vector were registered.

## **Efficiency of exclusion nets against *Bemisia tabaci* (Homoptera: Aleyrodidae) and TYLCV incidence in greenhouse tomato**

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The introduction of the Tomato Yellow Leaf Curl Virus (TYLCV) and its vector, *Bemisia tabaci*, to Morocco has limited tomato production from early season disease transmission that have significantly reduced yields in many regions. In response growers rely on the use of exclusion nets to prevent *B. tabaci* adults carrying TYLCV into the greenhouse. The most common mesh size currently in use is 10\*14 threads per cm<sup>2</sup>. However, field observations have indicated this mesh size may be insufficient at excluding *B. tabaci* adults as well as other whitefly species. Laboratory and field studies were initiated to evaluate the optimal mesh size for *B. tabaci* and *Trialeurodes vaporariorum* exclusion and examine the impact on tomato management practices. Laboratory experiments were carried out to test the efficiency of 3 mesh sizes, 10\*14, 10\*20 and 10\*22 against whitefly adults and their effect on two endemic important natural enemies, *Eretmocerus mundus* and *Diglyphus isaea*. In addition, field trials were employed to examine the effect of the 10\*14 and 10\*20 mesh sizes on whitefly numbers, TYLCV incidence and pesticide use. Laboratory evaluations revealed significant reductions in *B. tabaci* penetration for the smaller mesh sizes (77.5% penetration for the 10\*14; 6.83% and 0.0% penetration for the 10\*20 and 10\*22, respectively), and for *T. vaporariorum*, (74.2% penetration for the 10\*14; 0.9% and 0.4% penetration for the 10\*20 and 10\*22, respectively). Results also revealed no significant differences in penetration for *E. mundus* between the 10\*14 and 10\*20 (82.1% versus 72.9% penetration, respectively), but differences were significant between the 10\*22 and the other mesh sizes (48.2% penetration). For *D. isaea*, significant reductions were observed for the 10\*20 compared to 10\*14 (83.2% versus 49.5%, respectively). Season-long field trials demonstrated that under commercial conditions the 10\*20 mesh size showed fewer *B. tabaci* captures inside the greenhouse relative to outside captures. While the 10\*14 greenhouse interior captures were more similar to outside captures. More importantly, the 10\*20 showed significantly less TYLCV incidence relative to the 10\*14 greenhouse (100% versus 23.4% incidence, respectively). Further, the 10\*20 greenhouse required 7 fewer pesticide applications relative to the 10\*14 (18.5% reduction) while no differences in fungicide applications were observed. Our results demonstrated that the 10\*20 mesh size may provide more efficient exclusion of *B. tabaci* and other whitefly species and reduced TYLCV incidence with minimal impact on natural enemies and pathogens.

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## **Repellency and deterency caused by plant extracts on *Bemisia tabaci* adults**

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In Mesoamerica and the Caribbean, *Bemisia tabaci* acts mainly as a geminivirus vector of food crops, and insecticides have generally failed to provide effective control, as threshold damage can be as low as one individual per plant. Since the impact of viral diseases on yields is higher at earlier stages of plant development, any management scheme should focus on this critical early period, by minimizing contact between the vector and the host plant. For tomato, this may be achieved by transplanting virus-free seedlings produced in insect-proof structures, and later protecting them by chemical repellents/deterrents. Even though both cooking and mineral oils can deter *B. tabaci* in laboratory and greenhouse experiments, it is not known if plants can be protected once they are exposed to viruses in the field. Thus, using volatile repellents, which would preclude whitefly adults from reaching a plant, seems to be a more reasonable approach to prevent viral infection. So far, some 70 plant extracts, including both formulated commercial products and crude (hydroalcoholic) extracts, have been tested at CATIE. Extracts from the following 10 species have stood out for their ability to deter/repel adult whiteflies: worm-seed (*Chenopodium ambrosioides*), sword bean (*Canavalia ensiformis*), "chile muelo" (*Drymis granatensis*), bitterwood (*Quassia amara*), mother of cocoa (*Gliricidia sepium*), neem (*Azadirachta indica*), wild "tacaco" (*Sechium pittieri*), "sorosi" (*Momordica charantia*), fish bean (*Tephrosia vogelii*), and wild sunflower (*Tithonia diversifolia*). Their effect has been detected under greenhouse experimental conditions, at doses as low as 10 ml/l water (1% v/v), and it remains to be elucidated if they act either as deterrents or repellents.

## **Recent advances in host plant resistance to whiteflies in cassava**

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Host plant resistance to whiteflies is rare in cultivated crops. At the International Center of Tropical Agriculture in Colombia, more than 500 cassava clones have been evaluated for resistance to *Aleurotrachelus socialis*, the cassava whitefly. Several cultivars with high levels of resistance have been identified. When feeding on resistant genotypes *A. socialis* had less oviposition, longer development periods, reduced size and higher mortality than when feeding on susceptible ones. Several resistant hybrids have been evaluated by the Colombian Ministry of Agriculture and one has been selected for released to farmers. A mapping population was developed to identify the genomic regions responsible for whitefly resistance. Clones showing resistance to *A. socialis* are now being evaluated for resistance to *Bemisia tabaci*, biotype B. Initial results indicate that *B. tabaci* B collected in Colombia, South America, is not well adapted to cassava.

## **Effect of the glandular exudate of tomato genotypes on the development of *Bemisia tabaci* biotype B**

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*Bemisia tabaci* (Gennadius, 1889) is one of the most important tomato pests in Brazil, causing severe losses due to the sap suction, the induction of the irregular ripening of the fruit and the viruses transmission. The objective of this study was to determine the effect of eight tomato genotypes on the development of *B. tabaci* biotype B under greenhouse conditions, by using leaflets with and without glandular exudate. Whitefly adults were transferred to a voile cage, where they were allowed to oviposit for 24 hours. The variables evaluated were number of eggs, egg viability, incubation period, number of nymphs, duration of the nymphal stage and nymphal viability. Intact leaflets of LA0716 (*Lycopersicon pennellii*) were less accepted for oviposition than leaflets without exudate, which suggests an antixenotic effect. LA1584 (*L. pimpinellifolium*) increased the duration of the nymphal phase and reduced the viability of that phase, suggesting antibiosis. Antixenotic resistance in LA1739 (*L. hirsutum*) and PI134417 (*L. hirsutum* f. *glabratum*) was not associated to the glandular exudate. PI134417 reduced the nymphal viability, which suggests an antibiotic effect of that material on the insect. LA1739, in spite of reducing the oviposition, was suitable to the development of the insect. LA1609 was highly preferred for oviposition in the presence of the exudate, which can be associated to the highest pH found in leaflets of that genotype, however it reduced the insect survival.

## **Screening of tomato genotypes infested by *Bemisia tabaci* B biotype**

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A comparative study has been carried out in order to characterize resistance to *Bemisia tabaci* B biotype. Seventeen *Lycopersicon* genotypes were screened without environmental control in six greenhouses covered with plastic and anti-aphid. The wild genotypes were *Lycopersicon pennellii* (LA 716), *L. hirsutum* (PI 127826 and PI 127827), *L. hirsutum* f. *glabratum* (PI 126449 and PI 134417), *L. peruvianum* (CGO 6707 and CGO 6712), *L. peruvianum* f. *dentatum* (WYR 2020 and LA 111), *L. peruvianum* f. *glandulosum* (LA 1113 and LA 1113-2); the commercial genotypes of *L. esculentum* were the hybrids Buna VFN, Fortaleza, Debora Plus VFN, Carmen, and the cultivars Gem pride, Santa Clara. Trials were set up in a randomized block design with seventeen treatments (genotypes) and six blocks (greenhouses). Data were submitted to ANOVA and means were compared by Tukey test ( $P=0.05$ ). The wild access genotypes LA 716, PI 134417, PI 127827 and PI 127826 were the less preferred by the insect. On the other hand the genotypes LA 1113-2 and Bruna VFN hybrid were the most susceptible.

## **Scanning electron micrographs of description and capture of *Bemisia tabaci* biotype B on *Lycopersicon pennellii***

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The resistance in tomato to the genus *Bemisia* has been associated with the presence of leaf glandular trichomes or their exudates. Aimed to verified the *B. tabaci* biotype B capture by *Lycopersicon pennellii* trichomes, this work registered whitefly apprehend by leaflets using scanning electron microscope. Leaflets of *L. pennellii* were collected and placed in open Petri dishes kept in covered glass chambers (50 x 50 cm). Cotton wads moistened with 3% glutaraldehyde in potassium phosphate buffer at 0.1mol/l and pH 7.4 were previously distributed in the dishes. The leaflets were maintained in the chamber for 72 hours and were then post-fixed in osmium tetroxide vapor at 2%. The material was dried for 48 hours in silica gel desiccator mounted and metalized with 35 nm gold-palladium coating in a Denton Vacuum Desk II metalizer, and scanned (JEOL JSM 5410 scanning electron microscope, 15 KV). The scanning electron microscope showed the *L. pennellii* glandular trichome IV type is the important factor to the resistance because it functions like "whitefly-killer".

## **Non-preference for feeding and/or antibiosis effects of tomato genotypes on *Bemisia tabaci* biotype B**

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*Bemisia tabaci* biotype B became one important crops pest in Brazil since 1990, mainly tomato. Researchers study to find alternative form to control this pest, besides chemical control. The objective of this work was to determine the mechanisms of resistance (non-preference for feeding and/or antibiosis) through the biology of *B. tabaci* biotype B on tomato genotypes. Four wild tomato genotypes *Lycopersicon pennellii* (LA 716), *L. hirsutum* (PI 127826 and PI 127827), *L. hirsutum* f. *glabratum* (PI 134417) from the Cenargen/Embrapa germoplasm bank, and two commercial genotypes of *L. esculentum*, Bruna VFN hybrid and Santa Clara, was evaluated during winter and spring seasons. This assay consisted of plants placed in individual cage (60 cm x 40 cm diameter) covered by fine mesh nylon. It was released 100 adults whiteflies during 24 hours, and obtained one-day-old whitefly's eggs. After this period the numbers of eggs were counted, between 10 and 20 eggs per plant, in five plants per genotype. Eggs and nymphs were observed daily using magnifying glass (20x). The eggs viability, incubation period, nymphal period (based on the exuviae presence), life cycle and adult longevity were calculated. The trials were set up in a randomized block design with six treatments (genotypes) and five replications (plants). Data were submitted to ANOVA and means were compared by Tukey test (P=0.05). No genotype showed antibiosis mechanism.

## **Integrate management of whitefly and Sunscald: the major tomato yield reducing factors in the Sudan**

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Whitefly (*Bemisia tabaci* Gen.) and sunscald are the major yield reducing factors of tomatoes in the northern Sudan. The effectiveness of some cultural practices and insecticides were assessed in two field experiements. In the first season staked, unstaked, staked+grasscover, unstaked+grasscover, unstaked+mulch+grasscover and unstaked+mulch were evaluated. In the second season the cultural practices were integrated with chemical insecticide Malataf. Staking significantly reduced whitefly infestation as well as losses caused by sunscald. Staking also increased the yield of marketable tomatoes upto 95.3%. Losses caused by fungi were also significantly reduced by staking. Integration of cultural practices with Malataf can be a solution to all the year round production of tomato.