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Introduction

This issue of *THE ICAC RECORDER* has three articles. Genetically engineered cotton resistant to Lepidoptera insects is at final stages of testing and may be released for commercial cultivation in 1995. Herbicide-tolerant cotton may be available to farmers in 1998. Before such cottons are released for commercial adoption, they must undergo extensive field testing. However, societal concern regarding the non-laboratory testing of field crops involving biotechnology has increased in the past few years. Some countries have already formed guidelines for field testing of genetically engineered agricultural organisms, while others are in the process of doing so. A summary of the guidelines formed by the USDA is given in the first article.

In the second article on "Cotton Protoplast Culture," Dr. M-C. Peeters and Dr. R. Swennen, Laboratory of Tropical Crop Husbandry, Belgium, have reviewed the work done so far on protoplast culture in agriculture, with particular reference to cotton.

The third article discusses the occurrence and losses in cotton due to nematodes. Possible remedial measures to control nematodes have also been addressed.

A brochure on the International Cotton Research Conference, to be held in Australia in February 13-17, 1994, has been sent to all on the mailing list of *THE ICAC RECORDER.* To participate in the conference, please return to the ICAC the completed preregistration form given at the end of this publication.

Biotechnology: Guidelines to Field Testing in the US

The world's first field testing of transgenic cotton was conducted in the US in 1989. The genetically engineered cotton had a nonrelated gene transferred from the *Bacillus thuringiensis* endotoxin to larvae of Lepidoptera insects in general and *Heliothis* species in particular. Since 1989, trials on genetically modified cotton have expanded and many government organizations and private companies have become involved in the use of biotechnology on cotton. Conventional breeding methods have developed genotypes which have always been considered to be environmentally safe. Researchers had no conditions imposed on field testing the genetically modified genotypes. Conventional breeding research was never criticized as a form of environmental danger. Since the realization of the fact that genetic material in a distinct species can be recombined for purposeful and directed manipulation in agriculture, a need was felt to regulate the field testing of such organisms. Societal concern has increased in the last few years.

The US Department of Agriculture has developed a system of biosafety and has published guidelines for field testing of genetically modified agricultural products. An Agricultural Biotechnology Research Advisory Committee (ABRAC) has been set up to prepare guidelines and keep them scientifically current, technically correct and appropriate to the needs of both the public and scientific community. The participating institutes work through the Institutional Biosafety Committee (IBC) which is responsible for approving experiments in the specified categories. Above the IBC and ABRAC, the National Biological Impact Assessment Program also conducts research, shares information and facilitates monitoring of environmental releases of genetically modified organisms. The National Biological Impact Assessment Program also works to disseminate knowledge for safe field testing of the genetically modified organism. A permit from the Animal and Plant Health Inspection Service of the USDA is required for interstate movement, importation or the release into the environment of a plant pest or other regulated article for use in an experiment. If the test organism has a pesticidal effect, such as microbial pesticides or the release of sterile moths of various pests, a permit from the Environmental Protection Agency is required before proceeding with field trials. Under the guidelines set to test genetically engineered material, regulatory requirements and the responsibilities of the principal investigator (researcher), the participating institution, and the USDA have been defined to cover the risks, if any, arising from field testing of the new material.

Responsibilities of the Principal Investigator (PI)

The PI is a researcher who is responsible for planning and implementing the field testing of genetically modified agricultural products. The main responsibilities of the PI are as follows:

1. To determine whether the experiment is covered by the guidelines and to follow the recommended procedures of the guidelines. In case of any difficulty in the implementation of guidelines, it is the responsibility of the PI to bring such matters to the notice of IBC and the Office of Agricultural Biotechnology (OAB). He will also report any new information regarding guidelines to IBC and OAB.

2. The PI will make an initial determination of the conditions for safely performing research and appropriate levels of oversight, keeping in view the level of safety concern for the unmodified organism (on a scale from 1 to 5), effect of genetic modification on safety and the level of safety concern for the modified organism from 1 to 5.

3. To prepare a detailed description of the experimental area and surrounding environment and the specific confinement and other safety practices that will be used, including a detailed discussion of the risks and documentation supporting a determination that the research can be safely conducted under the conditions proposed.

4. The PI will decide and convey to the authorities the description of the experimental design, appropriate practices and techniques, as well as the quantity of the genetically modified organism to be used in the research. He is also responsible for submitting the subsequent changes to the IBC for review and actions.

5. To prepare a detailed description of how the genetically modified organism was produced, information supporting its molecular characterization,

the amount of inserted genetic information and location, and the stability of the genetic modification. The PI will also provide the anticipated effects of gene expression along with supporting documents.

6. To collect all scientific, common and trade names as well as all designations necessary to identify the donor organism(s), the recipient organism(s), the vector or vector agent(s), and the constituents of any product that contains the genetically modified organism proposed for use in the research. The PI will prepare a purposeful discussion of the objectives and possible benefits of the research under trial.

7. To prepare a description of monitoring procedures that will be used to detect the escape of the modified organism from the intended confinement and a description of contingency plans to be implemented in the event of an escape.

8. To make available to the research staff copies of the protocols that describe the practices to be used, particularly with respect to confinement practices. He will train and instruct the staff in field experimentation tech-

niques to be used in the trials, as well as precautionary measures, and deal with accidents.

9. In the case of testing in states other than the state of origin, the PI is responsible to contact the appropriate State Agriculture Authorities and adhere to all the state laws.

10. The USDA may require additional information other than what is already provided in the application for permission. It is the responsibility of the PI or other institute-designated individual to provide such information.

11. To report to the IBC, as soon as they are recognized, any research-related accidents that have resulted or could result in human illness, in unanticipated plant or animal disease, or in the case of an escape by the organisms under study from the intended confinement. The IBC should report such research-related accidents to OAB within fifteen days.

12. To comply with applicable shipping requirements regarding animal, plant and human health protection and policies, permit requirements and containment conditions for possession of certain organisms.

13. The PI will determine the confinement level appropriate to the particular level of safety concern for the modified organism as well as develop a safety protocol to meet this level of confinement. He will also determine the appropriate organizational level of safety review.

14. To supervise the research staff so as to ensure that required safety practices and techniques are employed. To immediately correct and report work errors and conditions that may result in unsafe situations or the inadvertent release of experimental biological material.

Responsibilities of the Institute

1. The establishment and implementation of policies that provide for safe conduct of research in compliance with the guidelines.

2. All institutes undertaking research involving biotechnology should establish their own IBC. In the case of nonexistence of an IBC at the institute, it is the responsibility of the institute to with IBCs of other institutes.

3. To provide assistance to the PI responsible for research and to assure him that the research being conducted is in accordance with the guidelines.

4. To provide identification of its IBC members by name, area of expertise and affiliation in a report to the OAB and prompt notification to OAB of any membership changes.

5. To establish provisions to make available to the public all information on experiments conducted at the institution subject to the guidelines, unless it contains confidential business information or unless its disclosure is prohibited by a state or federal law, and to make available a general description of material withheld.

6. To assure that the IBC reports promptly to the OAB any significant problems with implementation of these guidelines.

7. To assure that the IBC reports to OAB, within 15 days, any research-related accidents that have resulted or could result in human illness, in unanticipated plant or animal disease, or in the escape of organisms under study from the intended confinement

8. To establish and implement the policies including confirmation that the organisms used and the conditions of research are assessed in accordance with the principles of the guidelines.

9. To ensure that the PIs comply with the guidelines and applicable regulations and to assist them in doing so.

10. To ensure that the concerns of the community for the planned introduction of genetically modified organisms into the environment are solicited and addressed by the institution.

Responsibilities of the Institutional Biosafety Committee (IBC)

All institutes sponsoring field testing of agricultural research involving biotechnology are supposed to promote safe research. All such institutes must have their own IBCs or an affiliation with an IBC of other institutes. The IBC should have at least six members selected so that, as a group, they have expertise and experience for evaluating the biosafety and environmental effects of non-laboratory agricultural research involving biotechnology, as well as the use of recombinant DNA techniques. The composition of the IBC will be such that it has at least two members who are not affiliated with the institute, and who will represent the interest of the community. The IBC has the following main responsibilities:

1. To establish and implement policies that provide for the safe conduct of biotechnology research and to ensure compliance with the applicable guidelines.

2. To create and maintain a central reference file and library of catalogs, books, articles, newsletters, and other communications as a source of evidence and reference, including such items as the availability of safety equipment, the availability and level of biological containment for various host-vector systems, suitable training of personnel, and data on the potential biohazards associated with certain technologies.

3. To develop a safety and operations manual for safe conduct of research.

4. To certify, upon request, the safety of facilities, procedures and practices and that the training and expertise of the personnel involved have been reviewed and approved.

The PIs are encouraged to attend the IBC meetings. These meetings are also open to the general public, consistent with protection of privacy and propriety rights.

Responsibilities of the USDA

The USDA responsibilities include administrative review by the OAB, scientific review by the ABRAC and policy review by the CBA.

Administrative Review by the Office of Agricultural Biotechnology

The OAB has the responsibility to provide administrative support in coordinating USDA policies and procedures pertaining to biotechnology. Other responsibilities consist of the following:

1. To coordinate biotechnology activities within the USDA.

2. To provide executive secretarial and staff support to biotechnology committees.

3. To assist in the development and implementation of policies and procedures pertaining to the conduct of research and experimentation in biotechnology.

4. To maintain records of research and regulatory activities carried out under the biotechnology authorities of the Secretary of Agriculture.

Scientific Review by the Agricultural Biotechnology Research Advisory Committee

The responsibilities of the ABRAC are as follows:

1. To oversee the review of research projects that are subject to the research guidelines or subject to regulatory review, recommending action thereon to the higher authorities.

2. To evaluate the adequacy of information submitted by researchers to be used by the Department in research for environmental assessments.

3. To develop and recommend additions and alterations to research guidelines and protocols.

4. To provide advice when requested to other Federal, State and local agencies on agriculture-related research projects.

5. To provide information to and maintain communication with IBCs so as to ensure their capability in carrying out their agriculture-related functions.

Policy Review by the Committee on Biotechnology of Agriculture

The CBA is the primary instrument of the Department to review policies and programs on biotechnology. The responsibilities of the committee are as follows:

1. To provide advice, when requested, on initiatives, proposals, and policy for biotechnology-related product and article regulation and research, and to assist in the coordination of these activities.

2. To review scientific information submitted by agencies within the department.

3. To assist in identifying data gaps for basic research in agriculture biotechnology.

4. To foster public awareness of the scientific issues in biotechnology.

5. To provide departmental support for participation in the Biotechnology Science Coordination Committee, a committee in the executive branch, composed of members of each of the departments involved in biotechnology-related activities.

Regulation Requirements

More than one agency has regulatory authorities in the area of biotechnology. Most of the issues dealing with cotton, like pesticidal microorganisms, intergenic combinations, pathogenic organisms etc., involve the Animal and Plant Health Inspection Service of the USDA. Permits are required for field testing genetically engineered organisms and products that may be or may contain a plant pest. The permits are issued on a case by case basis. The following information is required to be provided in the application for the permit.

1. All details of the person who developed/supplied the material. Name, title, address, telephone number, signature of the person responsible for the trials and type of permit requested.

2. All scientific, common and trade names and all designations necessary to identify the donor organism(s), recipient organism(s), vector or vector agent(s), constituents of each regulated article which is a product, and, regulated article.

3. A description of the means of movement of the genetically modified material from one place to the other.

4. A description of the anticipated or actual expression of the altered genetic material in the regulated article and how that expression differs from the expression in the nonmodified parental organism.

5. A detailed description of the molecular biology of the system that was used to produce the regulated article.

6. The country and locality where the donor organisms, recipient organism, and vector or vector agent were collected, developed and produced.

7. A detailed description of the purpose for the introduction of the regulated article, including a detailed description of the proposed experimental or production design.

8. The quantity of the regulated article to be introduced, and the proposed schedule and number of introductions.

9. A detailed description of the processes, procedures and safeguards that have been used and will be used in the country of origin and in the US to prevent contamination, release, and dissemination or production of the donor organisms, recipient organism, vector or vector agent, constituent of each regulated article which is a product, and, regulated article.

10. A detailed description of the intended destination uses and/or distribution of the regulated article.

11. A detailed description of the proposed procedure, processes and safeguards that will be used to prevent escape and dissemination of the regulated article at each of the intended destinations.

12. A detailed description of any biological material accompanying the regulated article during movement.

13. A detailed description of the proposed method of final disposition of the regulated article.

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Cotton Protoplast Culture

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Introduction

Since World War II the predominant position of cotton as a textile fiber has been threatened by the development of synthetic fibers. In order for cotton to remain competitive, continuous fundamental and applied research is necessary to improve the quality of cotton fibers.

Intensive classical breeding efforts resulted in high yielding and high quality tetraploid cottons. Salt tolerance, insect and pest resistance, glanded plants with glandless seeds and climatological adaptations are objectives of many cotton breeding programs. Although a rich and useful genetic reservoir remains to be exploited in wild and wild relatives of cotton, further improvement through hybridization has become difficult because of incompatibility barriers.

These limitations resulted in alternative ways of genome improvement. Different techniques have been developed to transfer new genes into plants and the first transgenic cotton plants have been obtained. Detailed knowledge of the gene structure and regulation of its expression at the molecular level is necessary to introduce into the genome novel genes with interesting features. *Agrobacterium tumefaciens* is a vector system with the capacity for gene transfer to many plant species. This transformation technique has its limitations, however, because of its limited host range, low transformation efficiency, problems with bacterium removal and DNA manipulation in large plasmids. Alternative techniques for gene transfer such as biolistic or particle guns have created high expectations. Microparticles coated with DNA coding for a novel feature are accelerated and shot into cells or tissues. A detailed review on approaches and results of gene transfer into plants was recently published (Potrykus, 1991). The decisive step in the transformation process remains the regeneration of stable plants from the transformed cells.

Genetic engineering of cotton plants is currently the objective of many researchers. Fundamental information on the applicability of different techniques of gene transfer has been gathered and the first successful transformations have been achieved. Herbicide and insect tolerant transgenic cotton lines have been developed (Bayley et al., 1992; Perlak et al., 1990). Because of its superior regeneration ability, Coker 312 was used in these transformation experiments. Back-crossing is still required to incorporate the novel characteristics into commercial cultivars.

An excellent updated review on achievements and perspectives in cotton biotechnology was recently reported by Stewart (1991) and published in the series of ICAC review articles.

Objectives of Protoplast Culture

The above mentioned transformation techniques used cells as a source material. However, protoplasts are recognized to be ideal for gene transfer (Potrykus, 1991) because the cell wall is removed (no longer a physical barrier) and thus the plasmalemma becomes freely accessible. The frequency that genes reach and enter a protoplast is enhanced. The DNA uptake has become a physical process. Since no biological vector is required, the host range problem is circumvented. Electroporation and microinjection are gene transfer techniques considered for protoplasts.

Genetic improvement can also be realized through fusion of protoplasts resulting in somatic hybrids. Chemical fusion using polyethylene glycol and electrofusion are the techniques mainly used for protoplast fusion. Protoplasts also create the possibility of recovering plants from a single cell origin (no chimera) and of selecting clones with novel characteristics via somaclonal variation. Until now, plant regeneration from cotton protoplasts is the limiting factor for the application of transformation techniques on protoplasts.

Protoplasts are also the material of choice for fundamental studies. Cell organelles and constituents can be better isolated from protoplasts than from tissues. The plasmalemma of *Daucus carota* protoplasts has been isolated and characterized (Boss and Ruesink, 1979). Intact chloroplasts, vacuoles, mitochondria and nuclei have been isolated from protoplasts by an osmotic shock and gradient centrifugation (Wagner and Siegelman, 1975; Ohyoma et al., 1977; Tallman and Reeck, 1980). Protoplasts are also used to study cell wall synthesis (Mock et al., 1990). Absence of the rigid wall facilitates the chemical and physical analysis of the cell membrane (Reinert and Bajaj, 1977; Stafford and Warren, 1991) and the study of specific cell wall enzymes (Fry, 1988). Protoplasts are also used in very specific research programs. The metabolism of C4 plants was studied using bundle sheaf protoplasts of C4 plants (Edwards and Huber, 1978). Light and stomatal functions were studied on guard cell protoplasts (Zieger and Hepler, 1979). Somatic cell genetics can also be studied with protoplasts (Binding, 1986). The regeneration capabilities of differentiated cells and the genetic basis for loss or preservation of regeneration ability in the course of cell differentiation can be analyzed.

Current Achievements on Cotton Protoplast Culture

In order to use protoplasts for genome improvement, protoplast technology needs to be developed. Protoplasts or plant cells without a wall were originally obtained by mechanical isolation. Nowadays the protoplasts are obtained by enzymatic digestion of the wall using mainly cellulase and pectinase. Isolation is done in such a way that protoplasts are not damaged but retain their ability to synthesize a new wall, as well as divide and regenerate into intact and stable plants.

The first isolation and culture experiments with cotton protoplasts were reported by Bhojwani et al., 1977. Protoplasts were isolated from hypocotyl-derived callus of *G. hirsutum.* The protoplasts were cultured in liquid medium. The first divisions were observed after 6 days and resulted in the formation of colonies consisting of 25-30 cells maximum after 5 weeks.

Finer and Smith (1982) reported the culture of protoplasts from friable hypocotyl-derived callus of *G. klotzschianum.* Isolation efficiency was influenced by the callus age, incubation time in enzyme mixture, concentration of osmoticum and agitation speed during digestion.

Division was observed 3 days after isolation and multicellular colonies were formed after 2 weeks. The regeneration capability of protoplasts isolated from hypocotyl or young stem tissue seems to be more limited than the one reported for cotton callus protoplasts since cotton cotyledon-derived protoplasts formed microcolonies of only 2-3 cells in *G. hirsutum* and 5-8 cells in *G. barbadense* (Firoozobady and DeBoer, 1986). A high rate of cell wall regeneration and cell division of freshly isolated protoplasts is required for optimal plating efficiency and successful plant regeneration. Firoozobady (1986) demonstrated that the ability of cotyledon protoplasts to regenerate new cell wall and undergo division depends upon the stage of the cell cycle at the time of isolation, which is dependent upon the age and growth condition of the donor tissue.

Thomas and Katteman (1984) isolated protoplasts from callus obtained from anthers of *G. hirsutum.* The yield of viable protoplasts is greatly enhanced when protective agents are used in the enzyme mixture. Ca^{2+}, Mg^{2+} or certain amino acids prevented the spontaneous lysis of protoplasts in the presence of RNA contaminants in the cellulase enzyme preparation. Using these protection agents macroscopic callus was obtained after 3 weeks culture.

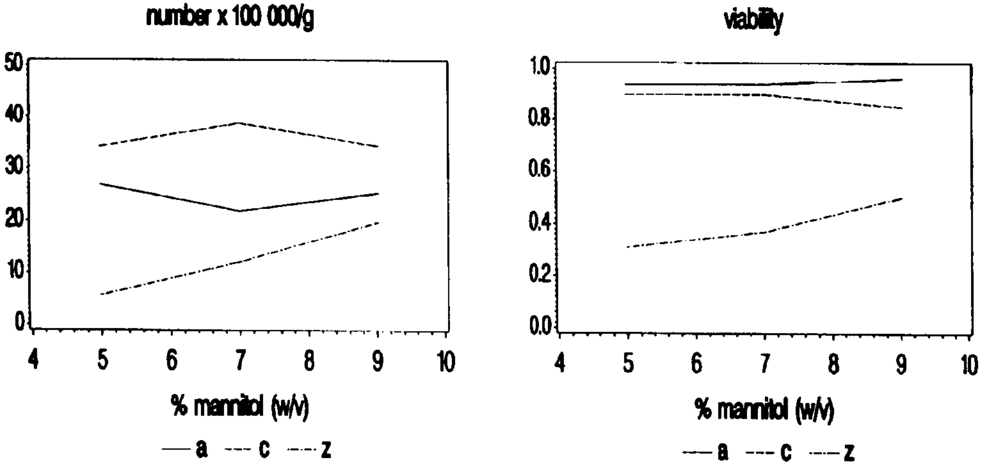
A method for the isolation and culture of protoplasts isolated from stem callus of *G. hirsutum* which leads to normally growing callus tissue was reported by Saka et al., (1987).

The highest regeneration stage obtained from cotton protoplasts is callus, and until now no reports have been presented on successful plant regeneration from cotton protoplasts.

In 1991 a research program, studying the genetic variability in cotton protoplast culture, was started at the Laboratory of Tropical Crop Husbandry with financial assistance from the Catholic University of Leuven (Belgium) and N.F.W.O. (Belgium). *G. hirsutum* cv. Coker 312 characterized by a high regeneration ability (Trolinder and Xhixian, 1989), *G. australe* a wild diploid and *G. hirsutum* cv. Zeta 4 a Greek commercial cultivar are being used in this study.

A fractionated factorial experiment was laid out. Factors recognized to be important in protoplast isolation were analyzed. These were osmotic conditions, incubation time, pH and enzyme concentrations. Yield and viability of cotyledon protoplasts of the 3 different varieties were assessed. Tendency diagrams demonstrate the variety-dependent influence of the considered factors. The influence of mannitol concentration on protoplast yield and viability is illustrated in Figure 1.

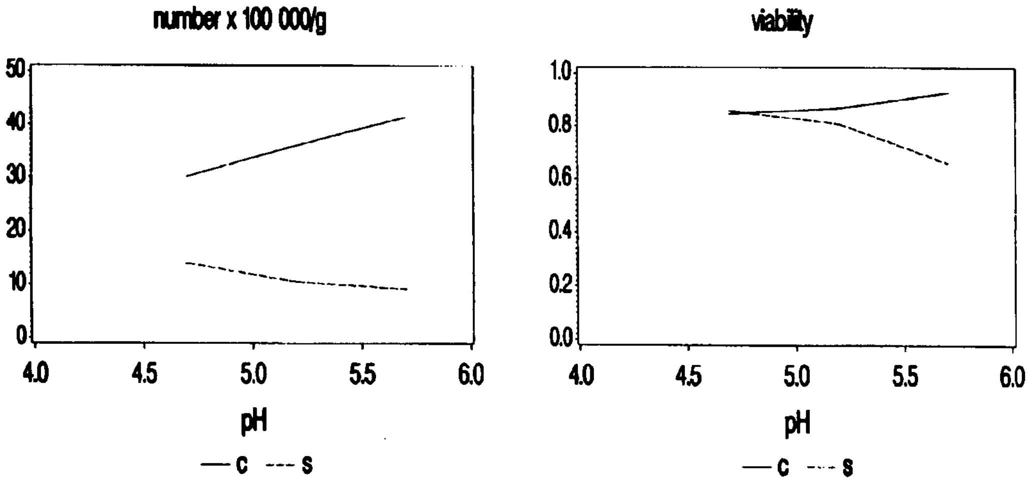
Figure 1: Influence of mannitol concentration on protoplast yield and viability (ratio of number viable protoplasts to total number) of *G. australe* (a) and *G. hirsutum* Coker 312 (c) and Zeta (z).



Results also indicated that protoplast characteristics such assize and chloroplast content depend upon isolation protocol. The average Coker 312 protoplast diameter of the different isolation experiments ranged between 20 and 27µm. The regeneration ability of these different protoplasts needs to be assessed.

Isolation conditions of cell suspensions and cotyledon-derived protoplasts of Coker 312 were investigated. Optimal conditions were different for both explants, suggesting that cell and wall constituents of cotyledon cells and cell suspensions are different. The influence of pH on protoplast yield and viability of both explants is illustrated in Figure 2.

Figure 2: Influence of pH on protoplast yield and viability (ratio of number viable protoplasts to total number) of cotyledon (c)- and cell suspension (s)-derived Coker 312 protoplasts.



Several reported techniques were tested for the further culture of Coker 312 cell suspension-derived protoplasts. Cell wall regeneration was observed within 3 days, but the first cell divisions were observed only after 2-3 weeks. This lagging phase was significantly reduced when a feeder layer technique was used. A highly friable callus was obtained after 6 weeks. Further regeneration is now in progress.

Since 1960, the FAO has promoted and supported network systems. In 1988 an Interregional Cooperative Research Network on Cotton was established with 15 participating countries; from Europe (Belgium, Bulgaria France, Greece, Netherlands, Spain and Turkey), the Middle East (Iran, Israel, Pakistan, Syria) and North African Regions (Algeria, Egypt, Morocco, Sudan). The objective of this network is to promote voluntary exchanges of information, material and experimental data in selected subject matter fields, as well as effective cooperation in research on mutually selected topics. During the 2nd Consultation of this Network in Thessaloniki, Greece, the results of our research program were presented. At the same meeting a new working group, "Biotechnology in cotton," was created and Mrs. Peeters of the Laboratory of Tropical Crop Husbandry was selected as its coordinator. Since cotton biotechnology in the participating countries is in its infancy, a call is made for a concerted action between the members and assistance from advanced cotton biotechnologists elsewhere.

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Nematode Management in Cotton

Nematodes are a limiting factor in cotton production in many countries of the world. According to the worldwide survey undertaken by Sasser and Freckman in 1986 the plant-parasitic nematodes are responsible for 10.75% loss in cotton yield. More than 30 genera and 128 species of nematodes are reported to be pests of cotton. But not all of them are harmful to the cotton roots. On a worldwide basis, the ten most important genera of plant-parasitic nematodes are *Meloidogyne, Pratylenchus, Heterodera, Ditylenchus, Globodera, Tylenchulus, Ziphinema, Radopholus, Rotylenchulus and Helicotylenchus.* Sometimes nonparasitic nematodes are present in the soil, but they do not show any effect on the plant. Out of these root-knot *(Meloidogyne incognita),* reniform *(Rotylenchulus reniformis),* lance *(Hoplolaimus columbus, Hoplolaimus galeatus)* and sting *(Belonolaimus* sp.) are of concern to the cotton growers. *Meloidogyne incognita* (race 3 and 4) is the most commonly cotton-damaging nematode in cotton producing countries of the world.

The nematodes, like weeds and other pathogens, occur in the field in the form of "hot spots," so sometimes they are there but are not detected in sample surveys. These hot spots are usually colonization sites for further multiplication and build up of the population. Similarly, the nematodes may be in small quantities, much below the threshold levels, and not capable of causing any damage to the crop. They are too small to be seen with the naked eye, but some species which do not belong to common groups can be seen without magnifying aids.

In Australia, nematodes do not pose any threat to cotton production. Soil and root samples were collected and studied during 1987/88 and 1988/89 from irrigated cotton fields of all cotton growing regions. The survey included fields which had a long history of cotton cultivation, so that if nematodes were present they could be detected. On the basis of two-year data it was observed that nonparasitic nematodes are commonly found in the samples analyzed. Parasitic nematodes were also detected, but their number was too low to cause any significant damage to the root.

In Pakistan, 19 genera of the nematodes have been reported to be isolated from cotton soils. Out of these, seven genera were parasitic, the most frequent being *Tylenchorhynchus* sp. and *Hoplolaimus* sp. with a population of 10.8 and 11.9 per 300 gram sample of sod respectively. The nematicides "nemagon" and "nemaphos" have been recommended for use on cotton soils for some time. But, owing to the unfavorable conditions for flare-ups of the nematodes, these nematicides were withdrawn from the market. Nematicides are neither recommended nor used by the growers. In 1986/87, after a long time, the attack of nematodes was observed in the southern part of the Punjab. The disease, occurring in the farmers' fields, could not be identified without the help of the researchers.

In the US, according to a survey covering 14 cotton-growing states during 1991/92, the average yield loss on account of diseases caused by nematodes is 2.4%, the maximum being 5.0% in Arizona, Georgia, and New Mexico. The most commonly occurring species are root-knot *(Meloidogyne incognita)* and reniform *(Rotylenchulus reniformis).* Climatically, 1991/92 was a good year for Louisiana; otherwise, according to the estimates, during normal years about 60% of the total area in Louisiana is treated with nematicides. This percentage is likely to increase. Reniform is on the increase in Alabama, and it is feared that if steps are not taken it might surpass the root-knot as the predominant nematode species. In South Carolina, where almost every second field has nematodes, *Hoplolaimus columbus is* the most commonly occurring species followed by *M. incognita.*

Nematodes Symptoms

Nematodes cause serious injuries to the root of the cotton plant, thus affecting its productive potential. The diseases caused by the nematodes affect the plant physically in two ways:

- Nematodes have a depressing effect on plant height. The symptoms usually appear at the time of first bloom. The plants do not attain proper height on account of shorter and smaller number of internodes. In the case of severe attack the leaves become pale in color. The symptoms are very similar to the shortage of nitrogen or the lack of photosynthetic activities. Under adverse conditions of attack the leaves, after turning yellow, start falling on the ground. *Belonolaimus sp., Rotylenchulus sp., Hoplolaimus* sp. and *Tylenchophynchus* sp. cause this type of effect on the plant.
- There are other parasitic nematodes which form galls on the roots. The galls block the flow of plant nutrients and disturb the transfer of food material from the root to the upper part of the plant The plant becomes

weak, shows wilting symptoms and cannot maintain its normal growth rate. The leaves remain green, but under severe attack, the roots start rotting, which results in leaf shedding. The most common nematode species causing root-knot *Meloidogyne incognita* shows these symptoms.

Losses due to Nematodes

Loss In Production

The nematodes are responsible for both quantitative as well as qualitative losses in cotton. The yield losses depend on the prevalence of the parasitic nematodes and the stage of the plant. Due to the setback of gall formation, stunted growth and yellowing of foliage cause the plant to become weaker and to have fewer fruiting points. As a result, production is drastically reduced. Smith et al (1992) have reported 25.7% loss in yield as a result of nontreatment of a nematode-infested field vs one treated with nematicide. Treated plots showed a higher percentage of fruiting forms located at the first reproductive position of each node than the untreated plots.

Efforts have been made to develop data bases that quantify the relationship between the preplanting nematode population and lint yield at harvest. In the presence of Fusarium wilt, which is usually associated with root-knot----the most commonly occurring species of the nematodes----it is extremely difficult to develop a straight-forward equation to determine the cost effectiveness of the nematode control. Not only Fusarium wilt but the overall array of factors that can influence the nematode-lint yield relationship appears so complex that confidence in an equation seems unlikely.

For some of the work done in the San Joaquin Valley of California, where cotton is often attacked by *M. incognita, an* effort has been made to develop a relationship between the preplant population of the pest and its effect on cotton yield. The following equation given in the form of a table shows that egg and juvenile counts up to 55 per 2.2 pounds of soil do not have any negative effect on yield. Soil samples were taken in the month of March before sowing and the variety grown was the Acala type. From the above table, it seems necessary to spray when the population level exceeds 150 nematodes per 2.2 pounds of soil. The decision to spray is, however, not straightforward, depending on the cost effectiveness of the chemical used to control nematodes, especially in the case of nematicides like 1,3-dichloropropene which are very expensive.

Effect of Root-knot *M. incognita* on Yield in Sandy Soil in California

M. incognita **eggs and juveniles count per 2.2 pounds of soil**	**Percent yield loss**
0-55	0
100	2
150	5
200	7
400	15
600	22
800	27
1000	32

Loss in Quality

Nematodes do not have a direct impact on fiber characteristics until their population has crossed the economic threshold level. Smith et al (1992) treated the nematode-infested cotton field with 1,3-dichloropropene at the rate of 7.4 gallons/ha and 14.8 gallons/ha. The fields had a known history of nematode existence. The nematode population was counted at pre-planting, during the growing season and after the harvest. They observed differences in micronaire, percent mature fibers, fineness and fiber length values of check and treated plots. Micronaire averaged 4.7 in the check and 3.3 in the high rate treated plot, thus showing a significant rise in micronaire value of the cotton coming from the untreated plot. The fiber length in the check plots averaged 23.9 mm as against 27.4 mm in the case of high-rate treated plots. Untreated plots produced a higher percentage of mature fibers. The possible reason responsible for the change in fiber characteristics of the untreated plots seems to be the early but moderate boll load, with fewer harvestable bolls produced beyond peak bloom which could bring down micronaire and maturity values. Fiber length might have been affected by ambient conditions arising from the stresses caused by root injuries. After harvest it was observed that the nematode population had decreased by 87.6% and 37.0% in the plots treated with high and low doses of Telone II respectively.

In other studies conducted by Veech during 1989 and 1990 at College Station, Texas, changes in some fiber characteristics were observed, but the differences were nonsignificant. The work conducted by Cook and Namken (1992) has also shown that the micronaire value is higher in the untreated plot vs the plots treated with nematicide or Temik. They also observed a lower seed index in the case of untreated plots, which may be due to a higher percentage of immature seeds in the untreated plots.

Multiplication of Nematodes

Nematodes cannot multiply easily in all types of soil. Soil texture also influences nematode pathogenicity; for example *M. incognita* is more injurious to cotton in sandy soils than in soils with high clay and loam texture. Soil texture is also responsible for the formation of patches of nematodes in consonance with the sandy patches in the field. All light soils like coastal sandy soils and river deltas are more prone to fixation of nematode population and their faster multiplication as well as their pathogenic effect on cotton roots.

High temperatures are injurious to many insect pests of cotton and similarly to nematodes. Summer temperatures exceeding 40°C have a lethal effect on the nematode population. Most of the nematode species become inactive in areas where atmospheric temperature goes above 40°C, and these areas usually do not have a nematode problem. Nematodes can multiply at a faster rate in soils that have a moisture content of 40-60% of the field capacity. This condition cannot exist for a long time in soils where the atmospheric temperature exceeds 40°C. Thus heavy soils in areas having a hot summer serve as an important inhibitory factor for the multiplication of nematodes. The climatic factors of precipitation and cloud-affected light interception have a favorable influence on multiplication of nematodes during the crop season.

Research has shown that the impact of potassium fertilizer on Fusarium wilt is dependent on the level of varietal resistance. If a variety is fairly resistant to Fusarium, the addition of K will improve its capabilities to resist the pathogen causing wilting. Because nematodes, particularly the most commonly found nematode causing root-knot, occur in association with Fusarium wilt, the addition of potassium fertilizers will help to check the incidence of wilted and dead plants on account of nematodes only in the case where the variety has a fair resistance to nematodes.

Nematode Control and Prevention

It is extremely necessary to make sure that the nematode problem exists in a particular field before any control measures are adopted. It is also important to check the distribution and magnitude of the problem. Proper record-keeping of the infested fields can help make a cost effective decision for the control of nematodes. Once it is established that a particular field has the problem, soil analysis before sowing or during the season should be done to confirm the need for remedial measures. The available options are the use of nematicides, resistant or tolerant cultivars and cultural operations, used either alone or in integration.

Use of Nematicides

Chemical control of the pathogen has been in use for over 30 years. The nematicides may be soil fumigants of nonfumigants. Soil fumigants injected into the bed before planting provide effective control. The most commonly used nematicide is 1,3 dichloropropene (Telone 11). It is expensive but very effective at high levels of infestation. It has been observed that application of 1,3-dichloropropene at the rate of 15 gallons per hectare protects not only the current crop but also has residual effect on the following crop because the low population of nematodes multiplies at a much slower rate. Metham-sodium is another nematicide with partial fuming action. It has to be applied through drip irrigation so that it reaches the treatment zone with water.

Low infestations can be controlled with nonfumigant nematicides. The non-fumigant nematicides provide early protection to the cotton root, but a long season effect cannot be obtained, especially in the case of severe infestation. Some organophosphates such as "aldicarb" are also available in the form of systemic nematicides. They can be applied preplant, at planting in the seed furrow, or in 6 to 12 inch bands with soil incorporated over the furrow. The combination of in-furrow and side dressing has shown better control than either application method alone, but timing of the side dressing must be based on nematode soil populations rather than plant development stages.

Temik has shown to diminish significantly the population of nematodes at an early stage. Temik is an insecticide which is applied in a granular form at the presowing stage to get rid of early-season sucking pests like jassids, thrips and whiteflies. But it has shown a strong effect on the mortality of nematodes and has thus increased lint yield significantly over the check - another reason for low use of nematicides.

Varietal Tolerance

It has already been stated that most of the nematodes, particularly the common genus *M. incognita,* occur in the presence of Fusarium wilt Thus it is difficult to isolate the tolerance to nematodes offered by the cultivars in the form of increased yield from the increased yield due to higher tolerance to Fusarium wilt. Yield potential differences among varieties is another factor mixed with the complex of Fusarium and nematode attack. Efforts have been made to distinguish the effect of nematode pathogens on the basis of gall formation and differences in the population densities at crop maturity. Resistance to the wilt pathogen was assessed on the basis of the presence of vascular browning. Cultivar testing in nematode infested cotton fields have shown significant differences in lint yield among genotypes. A lot of work is going on worldwide to screen resistant varieties. Some Acala cultivars have shown better tolerance, but they do not eliminate the need for use of chemicals under high infestation. Resistant crops are available, but there is a greater need for resistant cultivars in African and Central American countries.

Greenhouse studies have been undertaken by McPherson and Jenkins (1992) in Mississippi to understand the basis of tolerance. Some possible effects of tolerance could be reduced penetration of juveniles, failure to undergo molts or development of juveniles into males. Methyl bromide fumigated soil in pots was inoculated with 10,000 eggs of *M. incognita* (race 3). The nematode development stages were stained at each six day interval starting from sowing. They observed that a lower number of juveniles penetrated into the moderately tolerant cultivar and few of them molted as well, but the majority of them molted into males. An even higher number of juveniles penetrated into the roots of the highly tolerant cultivar, few molted but only one of them was a female. This experiment shows that the tolerance in the cultivars does not limit the infestation rather the mechanism of tolerance is different. Moderate and high tolerance in cultivars is determined on the basis of the ability of the penetrated juveniles to molt and form females.

Cultural Control

Cultural measures are a long term method of nematode control. Crop rotation is the most convenient and effective method manipulating the nematode population. Nonhost crops do not permit easy multiplication of nematodes, reduce density and render the soil less damaging for the following crop. The useful included alfalfa, resistant cultivars of tomato, cowpeas soybeans and small grains grown in winter and spring when root-knot is mostly inactive. Corn and sorghum are also said to be resistant to *M. incognita* and give normal yield in a nematode-infested field, but they are susceptible hosts and do not help to suppress the population of *M. incognita.* It is very important to establish the population densities and damage functions before a multi-season crop rotation is planned.

A weed free summer, subsoiling or deep ripping and soil solarization also have depressing effects on the population of nematodes. Integration of these operations with host plant resistant cultivars and fumigation could give economical, sound and long-term control of nematodes.

There is a need to explore new methods to check and control nematodes if agriculture is to become more efficient and sustainable. New developments in the field of biotechnology might offer a more effective and precise control. The tools of molecular genetics are being used to identify if microorganisms and viruses on the basis of their DNA. Some microorganisms have shown the potential to protect plants against pathogens. Biocontrol strains have been isolated from cotton leaves, squares and roots and are under trials for future use. These organisms must establish a specific ecological nitch where they must eventually function to inhibit the target pathogen. So far naturally occurring microorganisms have been used to control the pathogen, but there is a need to search for recombinant DNA technology for enhanced activities. There is also a need to accelerate research activities to ascertain the basis of host plant resistance and incorporate it into commercial cultivars.

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Short Notes

- Kerley Ag, Inc. has developed a new potassium fertilizer for foliar application. The fertilizer, called KTS, has 25 percent K_2O and 17 percent sulphur in the form of Potassium thiosulfate (0-0-25-17). It is claimed to be an excellent fertilizer for in-season correction of potassium deficiency. It is available in the form of a clear liquid which is chloride free. It can be applied through a wide variety of situations flood irrigation, drip irrigation and sprinkler irrigation. KTS can safely be blended with many other fertilizers for use as a foliar fertilizer. It is compatible with urea solutions, in any ratio, for use on cotton. The product is available on a commercial scale. (Source: *The Cotton Gin and Oil Mill Press,* May 30,1992)

- It was reported in Volume IX, No.4 of *THE ICAC RECORDER* that a team of scientists at the University of Washington's Institute of Biological Chemistry has discovered polypeptides in plants which act as a messenger to warn the plant against pest attack or other physical injuries. The same team has now identified a gene that codes for a hormone and tells the plant to produce chemicals which help the plant defend against insects, disease or physical injury. The gene discovery is a step forward in understanding the signaling process of plants to confer host resistance against outer injuries. It is observed that discovery of the polypeptides, as a part of the warning system coupled with the genes which produce chemicals that help the plant resist pest attack, will form an important component of the understanding of host plant resistance in cotton. These achievements hold the potential that one day scientists may be able to alter the plant genetically so that it has a stronger signaling system that can produce pest resistant chemicals in greater quantities, enabling them to defend themselves better than today. (Source: *Ag-Biotechnology News,* May/June, 1992)

- Fiber qualities are adversely affected by drought conditions. Water shortages not only restrict plant growth and affect yield, but also damage the quality of fiber. Water stress conditions reduce fiber length and enhance micronaire value. Fiber strength is increased, but fiber elasticity is badly affected thus increasing fiber breakage during processing. Genetic tolerance to drought conditions varies with the genetic make-up of the plant, but only testing under field conditions shows the ability of the plant to resist water-stress conditions. Lately, Texas A&M University researchers at the

Blackland Research Center in Temple have discovered a technique called "carbon isotope discrimination" that may help to identify strains of cotton that can survive drought and produce more and better lint. The technique determines the ratio of two carbon isotopes in plant leaves: Carbon- 12, which is the most common carbon isotope in the atmosphere used mostly in photosynthesis, and Carbon- 13. Under water-stress conditions, plants with higher Carbon- 13 have been found to make better use of water, thus least affecting yield. Surprisingly, some plants with higher Carbon-13 also perform better when water is ample. By calculating the ratio of Carbon-13 to Carbon- 12 it would be possible to identify the drought-tolerant plants at an early stage, thus assisting in their selection and use in breeding for drought-tolerant conditions. (Source: *The Cotton Gin and Oil Mill Press,* June 27, 1992)

- Nitrogen is a major source of plant nutrition not only because it is required in maximum quantity, compared with other nutrients, but because plants cannot grow and survive without it. The symptoms of nitrogen deficiency usually appear in the form of yellow leaves and retarded growth. The quantity of nitrogen that should be added to the soil depends on its availability in the soil, soil type, requirement of the crop grown, climatic conditions and adjustment with other nutrients to keep a balance for normal growth. But the quantity of nitrogen needs to be estimated before the shortage symptoms appear, otherwise it is too late to make up the deficiencies. Different methods are used and some of them give the exact status of availability; however, none of them is efficient enough to satisfy the requirements of farmers who do not want to see the deficiency symptoms appearing in the field. Soil testing is not considered to be a successful and reliable method in areas receiving frequent rainfall, as there may be excessive and quick leaching of nitrogen. Petiole analysis is a more reliable method but requires destruction of plant material to obtain samples, takes several days to process samples and is also influenced by moisture level in the soil. Researchers at Auburn University, Alabama, have discovered a "Chlorophyll Meter" to determine the nitrogen requirements for cotton. The Chlorophyll Meter is small, handy, and easy to use in the field. A group of scientists at the University began a study to see if chlorophyll measurements in a leaf could be correlated with the nitrogen application rates in the soil. The preliminary results have shown that such correlation does exist and that the green color of the leaf can be used as a management tool for adjusting fertilizer doses. The Chlorophyll

Meter reads greenness of the plants and provides instant information which has shown distinct correlation between nitrogen needs, yield and meter readings. Once the correlation factors are established through further studies, the Chlorophyll Meter can be used to adjust the nitrogen fertilizer doses accordingly. Precise and timely application of nitrogen will help to increase yield, save money for the grower, protect quality characteristics of lint and minimize undesirable release of nitrogen in the environment. (Source: *Cotton Grower,* July 1992)