Opinion of the SCP on Bt - Resistance monitoring (opinion expressed on 4 March 1999)

Background

1. In December 1996, on the basis of the opinions of three Scientific Committees, the Commission adopted a Decision to place the Novartis Bt-maize on the market.. This maize is modified to express the insect specific Bt toxin in plant in order to control infesting Lepidoptera larvae when they consume the maize tissues. Following the specific recommendation of the Scientific Committee on Pesticides, the Commission decided to launch a programme to monitor for the development of insect resistance in target pests of the Bt-maize. An Expert Group on monitoring insect resistance to Bt was therefore established by the Competent Authorities under Directive 90/220/EC to produce a suitable monitoring protocol. The resultant draft Protocol for the monitoring of European corn borer resistance to Bt-maize (Document XI/157/98) was forwarded to the Scientific Committee on Plants for its scientific opinion. A sub-group of experts met in January 1999 to discuss the draft opinion and the original protocol.

Draft Monitoring Protocol

2. The salient features of the original monitoring protocol proposed by the Expert Group for each geographically distinct population of European Corn Borer (ECB) are to

· establish the baseline susceptibility to the insecticidal protein in insects derived from the field

(Field collection of 5th instar larvae from infested maize stalks in 4-5 fields where neither Bt-maize had been grown previously nor Bt-spray used before. Rearing until the next generation, then 1st instar larvae tested in the laboratory at a range of doses of Bt calculated to cause mortality between 10% and 90%. The results are subjected to probit analysis.)

· detect change over time in baseline susceptibility

(Collection of 5th instar larvae every 4 generations in the field to produce the next generation for 1st instar testing in the laboratory. Comparison with baseline by calculation of resistance ratios.)

· forecast the development of resistance to the insecticidal protein

(Rearing 4 subsequent generations by testing the 1st instar larvae of the first 3 generations in the laboratory with a dose calculated to cause 70%-80% mortality. Testing the offspring of the 4th generation selected in the laboratory with a range of doses to compare with original baseline data using resistance ratios.)

Comments of the Scientific Committee on Plants on the Draft Protocol

3. Since any resistance genes are likely to be present in the population at very low initial frequency, an effective monitoring programme must be sufficiently sensitive to detect the early development of resistance resulting from changes in gene frequency. This requires large

sample sizes for testing and an adequate understanding and interpretation of baseline data derived in the laboratory. Although the draft monitoring protocol requires the collection of 300 to 1000 5th instar larvae by collecting 100 maize stalks per field in 10 different fields, it may be better to sample ECB by collecting eggs from the surface of maize plants rather than 5th instar larvae which have already been exposed to the Bt plants. However care should be taken when sampling and interpreting the results since individuals emerging from the same egg batch are genetically related.

4. The extent to which baseline levels can be determined and interpreted will be influenced by the inherent variation in the responses of insect populations to insecticides and the likely variability in the test assays. The draft protocol does not indicate how it should be implemented in practice or how the selection of sites will be related to selection pressure in the field which will differ according to the extent of Bt-maize grown in any location. In addition, exposure of larvae in the field may vary due to late season senescence or to a reduction of toxin concentration (loss of titre) in certain transgenic maize hybrids.

5. Whilst the proposed protocol may produce resistant insects within 4 generations in the laboratory as a result of low selection pressures, it is not known how relevant this is to the situation in the field where insects will normally be exposed to a high dose in the transgenic plants. For practical reasons it is not possible to sustain insects in the laboratory at Bt concentrations equivalent to those expressed in the plant in order to mimic selection pressure under field conditions.

6. As a technique in the laboratory, the determination of resistance ratios is not as effective in detecting resistance or as sensitive as the application of a high discriminating dose (for example LC_{95} [1]). Selection for resistance can alter the slope of the dose-response curve as well as its location on the dosage axis. The level at which the discriminating dose is set should be based on field data of susceptibility in specific areas.

General Comments

7. The general issue of monitoring to detect the appearance of resistance to Bt in primary pests of Bt-pesticide plants together with management strategies to delay the onset of resistance, have been the subject of considerable discussion, debate and scientific publication, particularly in North America. This is associated with the earlier commercial introduction of GM crops in the USA. Although only key references are listed below, each contains a more extensive list of relevant published papers.

8. Out of the international debate there is a consensus view, shared by the Scientific Committee on Plants, that one of the most important elements of the husbandry of Bt crops is to develop and implement effective resistance management plans which will delay the appearance of practical resistance to Bt in the target pests. Any monitoring programme should relate directly to the resistance management plan and be targeted to areas of higher selection pressure in order to have maximum chance of detecting resistance at an early stage. In this case this should clearly relate to the areas and intensity of Bt-corn planted.

9. The resistance management strategy that is most widely accepted is the high dose/structured refuge model which is being implemented in North America. The intention is to kill all susceptible insects and heterozygotes that carry one copy of any resistance gene, and the inherent assumption is that this is achieved by a high level of expression in Bt-plants.

Structured refuges are defined as all suitable non-Bt host plants for the target pest that are planted and managed by people and should be maintained in proximity to Bt-plants. The principle is that an overwhelming number of susceptible pest adult insects emerging from the refuges will mate with any potential resistant insects from nearby Bt crops thus maintaining low resistance gene frequency. How large these refuges should be is a matter of considerable discussion. A conservative approach is recommended in the early stages of commercial experience. For example current recommendations in North America are that 20-30% of the maize area grown should be planted with non-Bt plants and where alternative spraying of pesticides to control ECB is anticipated then the refuges should be increased to 40%. The size and spatial arrangement of refugia should be investigated on an experimental scale since random mating may not necessarily occur in practice due, for example, to asynchronous emergence of sexually mature adults. As research findings and practical field experience increase such initial recommendations of refuge size may be modified (either decreased or increased). With the progressive introduction of Bt-crops into Europe, in practice there will initially be very large refuges (i.e. alternative non-Bt host plants) adjacent to planted GM crops.

10. It is recognised that the impact of predatory or parasitic beneficial insects from adjacent refuges adds another important layer of complexity in resistance management which is poorly understood. The functional ecology of such beneficials in relation to transformed crops in the field should be further investigated.

11. Monitoring to detect the development of resistance by field collection and subsequent laboratory tests is important. However the quality of the data generated should justify the effort expended. In some cases extended laboratory studies may be more relevant to field conditions dependent on the local biological cycle of the ECB and where high plant expression of the toxin in tissues may not be maintained all season. F_2 screening may prove a useful technique for monitoring incipient resistance development and is considered more likely to detect recessive resistance alleles than a straightforward discriminating dose assay: insects which have been collected in the field should be bred in the laboratory and their F_1 progeny allowed to cross breed before the F_2 larvae are tested with, for example, a discriminating dose.

12. Whilst alleles for partial resistance may be reasonably common in natural populations of the ECB, in contrast alleles for complete resistance (to high levels of toxin expressed in transgenic plants) may be rare. Laboratory testing could be enhanced by a standardised bioassay on transgenic plant material to distinguish between 'true' and 'partial' resistance (i.e. when larvae can survive to the second instar on toxin-containing diets but would not survive the high dose on transgenic plants).

13. Long term resistance management plans should also consider alternative tactics in combination with the transgenic expression of insecticidal toxins, as well as effects on minor pests and non-target organisms.

14. There is a strong argument that the Mediterranean Corn Borer (MCB) **Sesamia nonagrioides** should also be included in monitoring plans to detect the development of resistance to Bt. MCB currently affects at least 600,000 ha of maize in southern Europe (Southern France, all Spain, Portugal, Greece and southern Italy). When present it is more abundant and causes more damage than ECB. Several features of its biology suggest that MCB is as much a candidate for resistance development as ECB given the right conditions:

- the species principally feeds on maize and sorghum, although it does feed on a few wild graminaceae and typhaceae;

- it does not disperse through the season but adults from overwintered larvae may move several km to colonise maize fields in the spring.

- female MCB are sedentary and probably move only when mated. Therefore females from non-Bt-maize fields (potential refuges) would rarely mate with males from Bt fields through the season.

Conclusions - Managing Insect Resistance to Bt Corn

· The draft protocol to monitor for resistance in European corn borer to Bt-maize should be linked firmly to field management plans on the ground. The proposed protocol which is based on experience and practical data should be broadened by the inclusion of more sensitive laboratory tests to detect low frequency resistance alleles and should include a suitable discriminating dose. Monitoring should be targeted to more extensive areas of planted Bt-maize where selection pressures may be highest for resistance development and procedures adjusted according to the GM variety grown (in relation to seasonal decline of toxin concentration).

· Effective high dose/structured refuge resistance management plans should be implemented for Bt-maize. Initially very large refuges may exist in practice as the total area of Bt maize will be small in Europe. However, plans should also take account of spatial structuring on a local scale.

· Growers should be required to survey their growing Bt-maize to detect unusual patterns of pest damage and ineffective control which should be thoroughly investigated by insect sampling and laboratory testing. Field survey should include the collection of plant material and the laboratory determination of expression levels in tissue, and the assessment of pest densities in the Bt crop and surrounding vegetation at the time of the problem.

Recommendations

1. Three phases are envisaged in a revised monitoring programme:

The regular monitoring of field populations to detect changes in susceptibility to Bt;

More detailed experimental testing in the laboratory over several generations bred from field collected insects in order to predict the likelihood of resistance developing;

Additional research is required on the basic biology of the pest and on method development to extend and increase the sensitivity of monitoring and predictive testing. The interactions of GM crops locally with other pest control strategies e.g.integrated pest management should be explored by modelling.

2. All geographic populations of ECB and MCB should be defined and identified. Minor pests for each region should be identified in case of later secondary pest outbreaks following declines in target species.

3. The susceptibility of target pests should be established for each geographically distinct field populations before the introduction of GM crops

4. The field locations and varieties of all transgenic Bt crops should be notified each year to enable the monitoring to be targeted towards high risk situations.

5. Each year field populations should be sampled from transgenic crops and their surrounding areas and tested to detect changes in susceptibility, with particular attention to transgenic crops grown in successive seasons and any areas of naturally-high resistance/tolerance.

6. To predict the development of resistance, insects should be collected from the field each year, selected for 4 generations in the laboratory. Resistance ratios, based on LC_{50} values, should be used to determine significant shifts away from baseline susceptibilities. A more sensitive discriminating dose bioassay (LC_{95}) can also be carried out, before (F_1) and after (F_4) of the laboratory breeding selection, in order to test for resistant homozygotes.

7. In addition, it is recommended that in cases where incipient resistance is suspected, or considered more likely to develop, a separate portion of the sample should be taken for F_2 screening. Thus it will be necessary to split the samples and test the F_1 generation (as above) and subject the remaining half of the samples to more sensitive testing in order to express the recessive alleles either at F_2 or over the next 4 generations.

8. Individual growers and the supply industry (e.g. seed companies) should be obliged to notify the Competent Authorities (at the end of each growing season) of any control failures which should have been thoroughly investigated for evidence of resistance.

9. While these transgenic crops are at an early stage of introduction into Europe, there is a clear and urgent need for further research studies on both ECB and MCB to investigate the baseline variability and stability of susceptibility to Bt.

10. Improved experimental protocols should be developed to provide validated methods for the laboratory and the means to identify high risk target populations in the field.

11. The refuge concept should be thoroughly investigated using experimental studies and theoretical modelling, using all available information from elsewhere outside Europe which already grow GM crops on the larger scale. In addition, investigations of the ecological impact of GM crops should assess the risk to non-target organisms both within and beyond the crop ecosystem.

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[1] Lethal concentration that kills 95 % of the susceptible population