Insight into resistance allele frequency

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Bollgard II is the cornerstone of integrated pest management (IPM) for the Australian cotton industry. Monsanto is committed to ensuring the longevity and ongoing stewardship of the technology and invests a large quantity of resources in a number of key areas to ensure all stakeholders, growers and the wider cotton industry can continue to benefit from this valuable product.

One of our key stewardship investments is in our Helicoverpa resistance monitoring program in which we collaborate fully with CSIRO to ensure the continued viability of Bollgard II.

The aim of the resistance monitoring program is to detect any increase in the frequency of resistance alleles to Cry1Ac or Cry2Ab. The frequency of resistance alleles to Cry1Ac has still not been determined due to the lack of field resistance alleles detected to date.

Prior to the commercial release of Bollgard II into Australia, CSIRO isolated an allele conferring resistance to Cry2Ab, thus establishing the existence of a background resistance level to Cry2Ab. CSIRO isolated one of the alleles and developed a laboratory colony resistant to Cry2Ab. This colony is known as SP15.

The frequency of resistance alleles to Cry2Ab can be estimated by the use of two tests – an F_1 test and an F_2 test. An F_1 test uses the CSIRO colony that is resistant to Cry2Ab (SP15) which is crossed with a field collected individual to assess the proportion of the progeny that carry the allele conferring resistance to Cry2Ab. Prior to 2007–08 season, the F_1 allele frequency had not increased (see Figure 1). In the past 12 months the F_1 test has indicated a significant increase in the resistance allele

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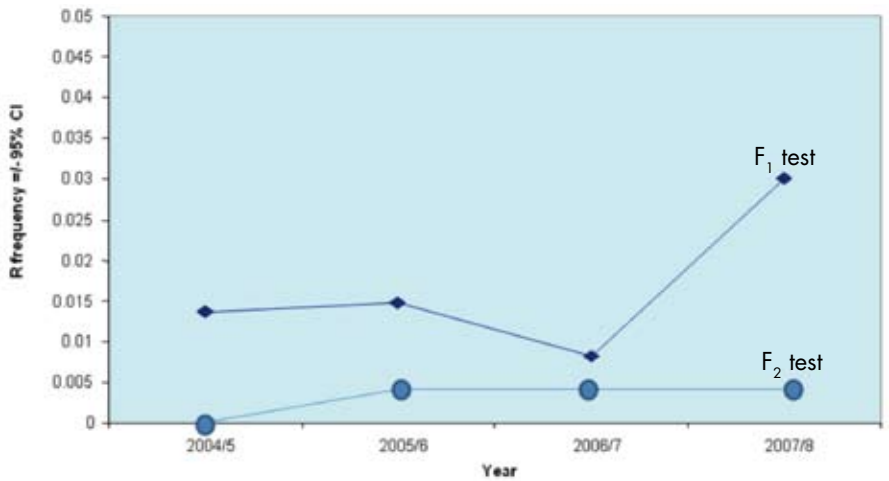
FIGURE 1: Change in allele frequency over time for the F_1 and F_2 tests



Acknowledging the contribution of Rod Mahon, Sharon Downs (CSIRO), Kym Deaves and Carolyn Johnston (Monsanto).

frequency for Cry2Ab, while the F_2 allele frequency has remained constant (Figure 1).

The F_2 screen is used worldwide, and is a method that estimates the frequencies of rare resistance alleles. The test is a cross between two field individuals (from the same collection) whose progeny are sibling mated and their offspring are screened with a discriminating dose of Cry2Ab. The presence of the allele that confers resistance to Cry2Ab in the F_2 progeny means that one of the crossed field individuals must have carried the allele. No changes have been observed in the frequencies of resistant alleles over the four years of F_2 screening for either protein (Figure 1).

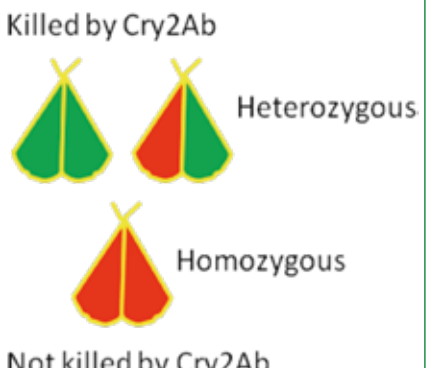
Monsanto and CSIRO are currently undertaking research to determine which test is reflecting the true allele frequency in the field.

The challenge for those working in this area is to understand why there is a difference between the two testing methodologies. CSIRO have looked at the possibility of there being a fitness cost, and found in their work that there is none (Mahon et al, in press). Monsanto is also conducting some experiments to determine if there is a fitness cost associated with the resistance allele at the time of mating – that is, when two males are competing to mate with a female, does a homozygous or heterozygous have as much opportunity to mate with her as does a susceptible male.

WHAT IS AN ALLELE?

In order to understand resistance to Bollgard II it is important to understand what an allele is. An allele is one member of a pair of genes that occupy the same position on a chromosome. Each moth carries two alleles and their offspring inherit one allele from each parent.

FIGURE 2: Genotypes of *Helicoverpa spp.* in the field



Cry2Ab resistance is a recessive mechanism

One of the key pieces in the resistance puzzle is that the gene that confers resistance to Cry2Ab is recessive, not dominant. This means that for the resistance characteristic to be expressed by an individual, it must be inherited from both its parents, not just one. When this occurs it is said to be homozygous resistant. When an individual carries only one of the alleles that confer resistance to Cry2Ab it is said to be heterozygous.

The resistant allele present in the heterozygous individual can still be controlled by Cry2Ab. That is good news! When a heterozygous individual mates with another heterozygous individual or a homozygous individual there is the potential for progeny carrying both resistant alleles to eventuate.

This is why the control of emerging moths that may carry both or one of the resistance alleles is a critical part of the Bollgard II RMP. In our resistance monitoring we are measuring the frequency of individuals that are heterozygotes (Figure 2).

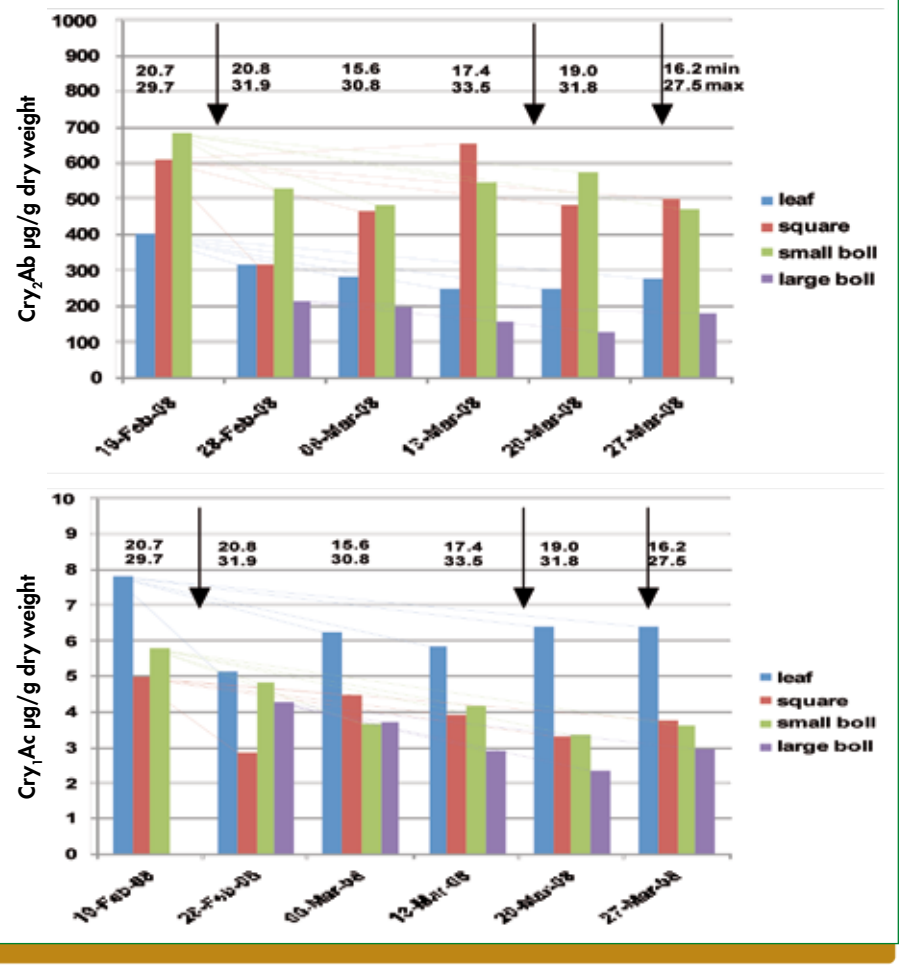
What does an increase in the frequency of resistance alleles mean?

An increase in resistance allele frequency means simply that the number of heterozygotes in the population has increased. Because there are more heterozygotes, there is a greater chance that they will mate and produce homozygous or 'resistant' offspring. Although this may occur there has been no recording of any cross-resistance such as an individual being resistant to both proteins. This means that Bollgard II will still control larvae that are homozygous resistant to Cry2Ab.

While 2007 data for the F_1 test did indicate an increase in the resistance allele frequency to Cry2Ab it is important that we assess the result again at the end of this season. Importantly, last season's results do not equate to field resistance and may not necessarily reflect the true field allele frequency.

Over the past couple of seasons larvae have survived in Bollgard II especially in the St George region. Both Monsanto and CSIRO have made collections of surviving

FIGURE 3: Results of some protein expression work undertaken on later planted cotton in St George in 2007–08 over the flowering period



larvae and run them through the resistance monitoring screens. Our results have shown that survival is not associated with resistance. The allele frequency of survivors is the same as the randomly collected Helicoverpa that we test.

In Figure 3 are the results of some protein expression work undertaken on later planted cotton in St George in 2007–08 over the flowering period. The arrows denote irrigation date. Monsanto has set up season long expression testing for both Cry1Ac and Cry2Ab for the 2008–09 season at two properties in the St George growing area, to attempt to quantify any environmental impact on protein expression. Monsanto has previously never been able to establish this effect, despite some anecdotal evidence.

THE IMPORTANCE OF STEWARDSHIP

An increase in the frequency of resistance alleles to Cry2Ab highlights the importance of the stewardship of Bollgard II technology.

Pupae busting is also a critical part of the Resistance Management Plan.

All growers of Bollgard II must ensure they have a well managed refuge that is attractive to Helicoverpa throughout the cotton growing season. The more susceptible moths that a refuge produces will decrease the likelihood of two heterozygote moths mating.

Pupae busting is also a critical part of the Resistance Management Plan (RMP) and must be performed correctly and in a timely manner. Resistance is a heritable genetic trait and so can be passed on from one generation to the next. Bollgard II cotton kills susceptible Helicoverpa and so any survivors of a Bollgard II crop will have survived on plant tissue not containing Bt – such as conventional volunteer plants, weeds or pollen from Bollgard II. As most susceptible Helicoverpa will have been controlled in a Bollgard II field, there is a significantly higher risk that any resistant individuals surviving will mate with each other.

Pupae destruction will reduce the potential population of moths that may emerge following a Bollgard II crop and therefore potentially reduce the risk of resistance accruing. Remember one pupa per square metre left to emerge can result in 10,000 moths emerging per hectare, which could seriously impact on resistance development.

COMMITMENT TO RESEARCH AND STEWARDSHIP

As part of Monsnato's commitment to the stewardship of Bollgard II we are actively investigating several issues this season:

- The resistance monitoring program is underway for the season and we will continue to work closely with Sharon Downes and Rod Mahon from CSIRO.
- We are continuing our refuge assessments in irrigated farming systems and this year we are also adding some dryland scenarios to this research program. The original research that determined the refuges and their sizes was conducted pre-Ingard. It is important now that the cotton growing system has changed so much to reassess refuges to ensure that they are performing (such as producing large numbers of susceptible moths).
- At St George we will be undertaking some season long expression trials which will involve regular tissue collections. We did a couple of these trials last season but it was in the late planted cotton. We need to repeat them again during the normal growing season. Our aim is to assess whether or not there is a change in expression at a point in the season that allows Helicoverpa to survive.
- Another aspect of our research is to investigate where moths are originating from i.e: where the larvae are developing. Monsanto has developed a test that can detect gossypol in the moths' body. If a moth tests positive to gossypol it spent its larval stage on cotton. In 2007 we placed pheromone traps in the lower Namoi, the Gwydir and Darling Downs on farms that had cotton refuges (sprayed and unsprayed) to collect H. armigera. The aim of the work is to understand what proportions of moths are emerging from cotton refuges or surrounding crops. This research will be assessed in conjunction with the refuge efficiency data to give us a sound understanding of where moths are being generated.

Monsanto is committed to the stewardship of Bollgard II and is actively working with industry to ensure its sustainability. The Resistance Management Plan (RMP) is in place to ensure Bollgard II remains the cornerstone of IPM for the Australian cotton industry.