# Plant therapy

## Progress to combat the plant stresses caused by climate and other environmental factors could bring huge increases in crop yields, writes **Cath O'Driscoll**

**For the past two years,** agricultural researchers in Costa Rica have been carrying out an unusual experiment. Based at a 200ha research station in Matina in the Caribbean lowlands, researchers have been investigating the effects of a novel chemical cocktail called *Alethea* in invigorating the region's valuable cocoa bean plants.

Specifically, plants grown in the presence of *Alethea* are being tested for their ability to resist the onslaught of the notorious fungal infection known as frosty pod rot, says David Marks, chief technology officer of Preston, UK-based firm Plant Impact which makes *Alethea*. 'In a good year, frosty pod rot wipes out three quarters of the cocoa crop, in a bad one 100% of the crop is lost.' Until now there have been no effective treatments.

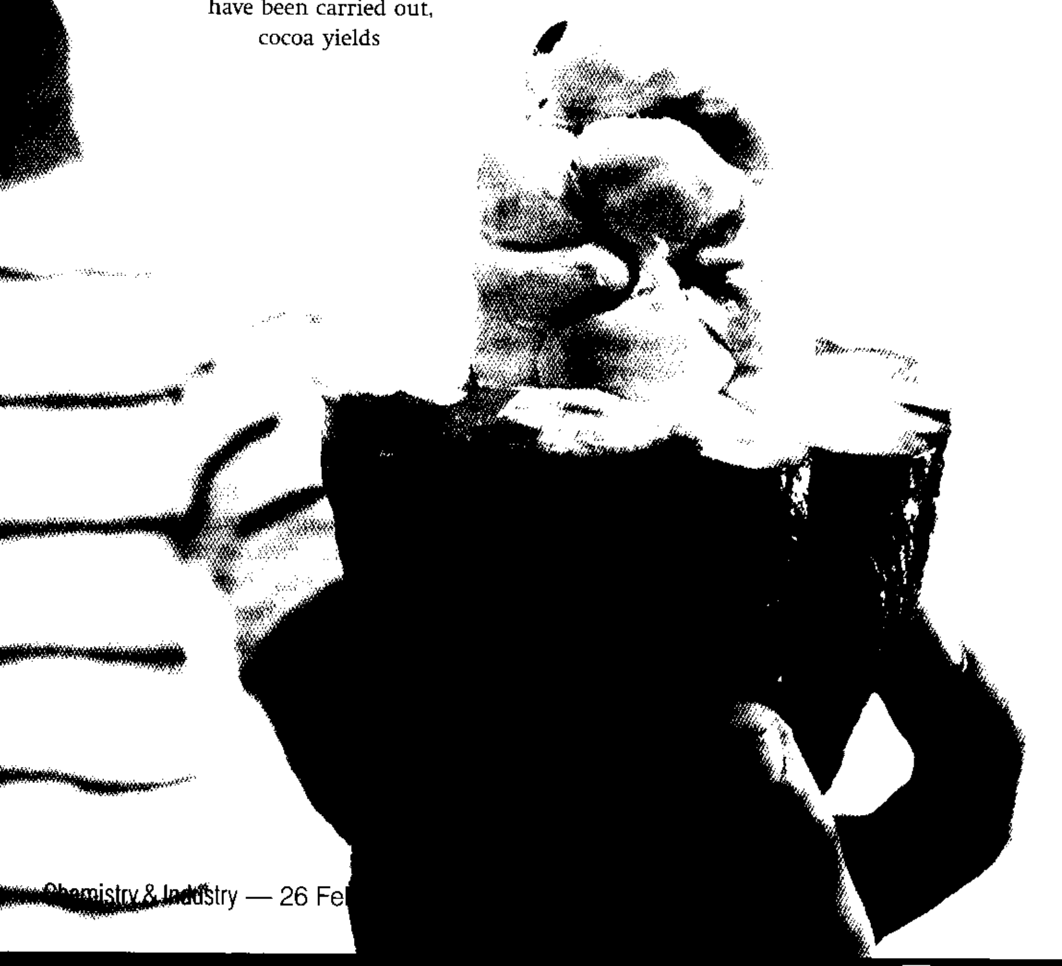
But all of that could be about to change. In the first two growing seasons in which the trials have been carried out, cocoa yields have increased consistently year-on-year by more than 50% when compared with the untreated plant controls, according to scientists at not-for profit organisation CABI who carried out independent field trials with the new ingredient.

'With cocoa you always have to be cautious because crop yields can be very erratic because one year the crop can be very high while in another it can do poorly,' says Ulrike Klauss, codirector of CABI Caribbean and Latin America. 'But a 50% increase is very exciting and if we saw that in the third growing season now under study this would be fantastic for farmers.'

It may be early days yet, but *Alethea*'s usefulness, its makers are hoping, may ultimately extend far beyond the humble cocoa bean plant. Stress tolerance, the mechanism by which *Alethea* is understood to exert its effects, is one of the most sought-after chemistries currently being pursued by plant scientists, and not without good reason.

### In brief

- **Abiotic plant stress damage could be responsible for four fifths of crop yield loss**
- **Research shows that the best selling insecticide confers a protective effect against stress**
- **Plant breeding is a very promising area of research into abiotic stress**
- **US corn losses due to drought cost $8bn/year**



Abiotic plant stress damage, caused by environmental factors such as intense light, ozone, herbicide damage, temperature stress, water stress, flooding and damage from heavy metals, is believed to be responsible for losses of up to four-fifths of some crop yields.

And as well as stunting plant growth and reducing crop yields directly, abiotic stress can also leave plants weakened and more vulnerable to other – so-called biotic – stresses, caused by attack by insects and other diseases including frosty pod rot.

In one US study cited by Plant Impact, for example, average yields of wheat harvested in a typical season were found to be just 13% of those achieved in a record year, while yields of five other major crops investigated, from barley to sugar beet, averaged a fifth to just over a third of their record yields (Table 1).

Actual yield losses due to abiotic stress, however, are hard to quantify, as the type and frequency of the various stresses are complex and unpredictable. While abiotic stress has the bigger impact on productivity, therefore, biotic stress has continued to garner the lion's share of the agrochemical industry's research budget.

| Crop | Record yield (kg/ha) | Average yield (kg/ha) | Average yield (% of record yield) | Average loss due to biotic stress (% of record yield) | Average loss due to abiotic stress (% of record yield) |
|---|---|---|---|---|---|
| **Wheat** | 14 500 | 1880 | 13.0 | 5.0 | 82.1 |
| **Barley** | 11 400 | 2050 | 18 | 6.7 | 75.4 |
| **Soya bean** | 7 390 | 1610 | 21.8 | 9.0 | 69.3 |
| **Maize** | 19 300 | 4600 | 23.8 | 10.1 | 65.8 |
| **Potato** | 91 400 | 28 300 | 30.1 | 18.9 | 54.1 |
| **Sugar beet** | 121 000 | 42 600 | 35.2 | 14.1 | 50.7 |

**Average and record yields: of some major crops** — **Source: Data from Bray *et al* (2000)**

But interest in the less fashionable abiotic stress chemistry is gathering pace. At Bayer CropScience, researchers claim to be making headway in two specific research projects into abiotic stress control. The first of these involves the findings of research on imidacloprid – the world's best-selling insecticide - which show that the agrochemical's effectiveness may not only be down to its insecticidal abilities, but also due to the fact that it also confers a special protective effect against stress, explains Wolfgang Thielert, head of research support in Bayer CropScience's insecticides business unit in Monheim, Germany.

'Even in the absence of plant infestation with insects, imidacloprid markedly improves the growth picture and also demonstrated positive effects on crop yields,' says Thielert.

Studies of barley and cotton exposed to moderate drought stress showed that plants treated with imidacloprid exhibited greater leaf growth, and were therefore able to generate more energy from photosynthesis to boost yields. One other surprising finding, Thielert says, is that 'plants treated with imidacloprid also produced markedly more endogenous proteins to defend themselves against fungal diseases.

To capitalise on these new findings, Bayer CropScience has recently launched a new imidacloprid formulation sold under the label *Confidor OTEQ Stress Shield Inside*. Designed to transfer the active ingredient more efficiently inside the plant, this new oil dispersion formulation also improves the retention and adhesion of the spray droplets by plant surfaces, and so gives superior rain fastness, Thielert explains.

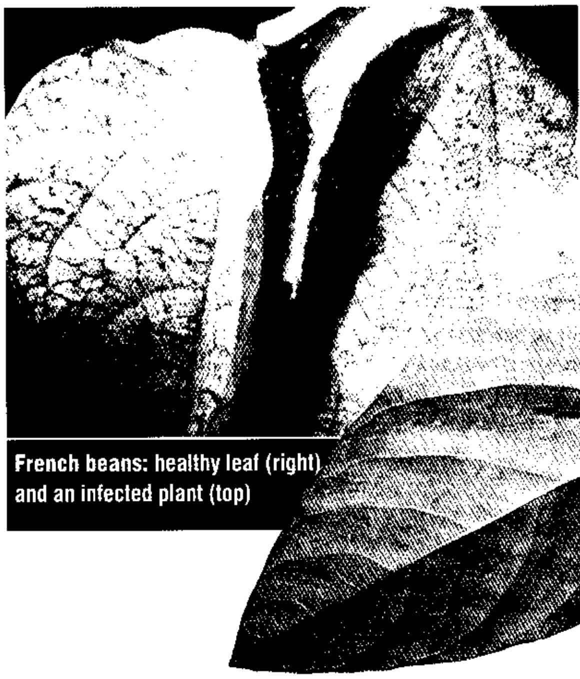
In the second of its abiotic stress projects, Bayer CropScience is using a more conventional approach to stress tolerance, by genetic manipulation. Specifically, says Michael Metzlaff, head of the crop productivity research group, work is focused on counteracting the loss of energy in stressed plants. Plants respond to stress by consuming large amounts of energy, he explains, thereby draining them of the power needed for vital physiological processes such as growth and carbon fixation in photosynthesis.

A key protein involved in this process, in plants as well as in all higher organisms including humans, is PARP (poly(ADP-ribose) polymerase), which controls the activity of several proteins involved in the stress response and so consumes large amounts of biological energy. Metzlaff and coworkers have now succeeded in developing oilseed rape varieties in which they have reduced the PARP activity to a level that, while still providing adequate stress protection, also offers significant energy savings.

Even during long periods of moderate stresses, when the control plants lost 50-80% of their normal energy store, PARP plants retained their usual energy levels, Metzlaff reports.

Interestingly, the team has been able to achieve this result by using a technology called RNA interference (RNAi). Notable for winning its discoverers last year's Nobel prize in medicine, RNAi technology works by employing a double stranded RNA sequence to 'silence' the gene coding for PARP, by seeking out and destroying its complementary messenger RNA sequence.

'Field trials in 2005 and 2006 have shown that oilseed rape plants with reduced PARP protein level are better able to withstand drought conditions,' Metzlaff says: 'In addition, field trial results for the summer 2006 harvest show a significant relative yield difference of between 20 and 44% measured as kg seeds/ha'. Bayer CropScience is now using its new RNAi-PARP technology in corn, cotton, oilseed rape and rice plants, with the aim of developing a new generation of stress-tolerant high performance crop varieties, which Metzlaff believes may be on the market within the next 10 years.



**French beans: healthy leaf (right) and an infected plant (top)**

*'Field trials have shown that oilseed rape plants with reduced PARP protein level are better able to withstand drought conditions'*

In yet other work, meanwhile, the company is also looking at the possibility of combining the *Stress Shield* effect of imidacloprid with new stress-tolerant plant varieties from the company's breeding research, as 'dual protection' against stress for crops.

Indeed, plant breeding, including GM plants, is currently one of the most promising avenues of research into abiotic stress, comments Antony Straszewski, chairman of the European Crop Protection Association's (ECPA) efficacy expert group. 'While healthier stronger plants may be more able to withstand stress, it is notoriously difficult to obtain consistent responses from plants when products are applied to try to alleviate the effects of the stressor.'

DuPont subsidiary Pioneer, for example, has been working on drought tolerance for more than half a century, says Marc Albertsen, director of agronomic traits and lead evaluation. The Iowa, US-based firm claims to have recently developed several corn plant hybrids with exceptional drought tolerance and high-yield potential, including one variety that can reduce water intake when needed. Each year it is estimated that one third of US corn acres will experience yield-reducing drought stress while, globally, the cost of corn losses due to drought is about $8bn/year, according to Pioneer market research.

Pioneer plans to launch a new transgenic trait post-2012 – pending successful performance testing, the company says. The goal is to increase yields by at least 25% over conventional corn during drought stress.

But genetic manipulation for stress tolerance is complex work. Plants' natural ability to cope with stress often involves multiple metabolic pathways regulated by more than one gene and experts agree there is unlikely to be any one 'silver bullet' capable of switching on a stress tolerant trait for all types of plants.

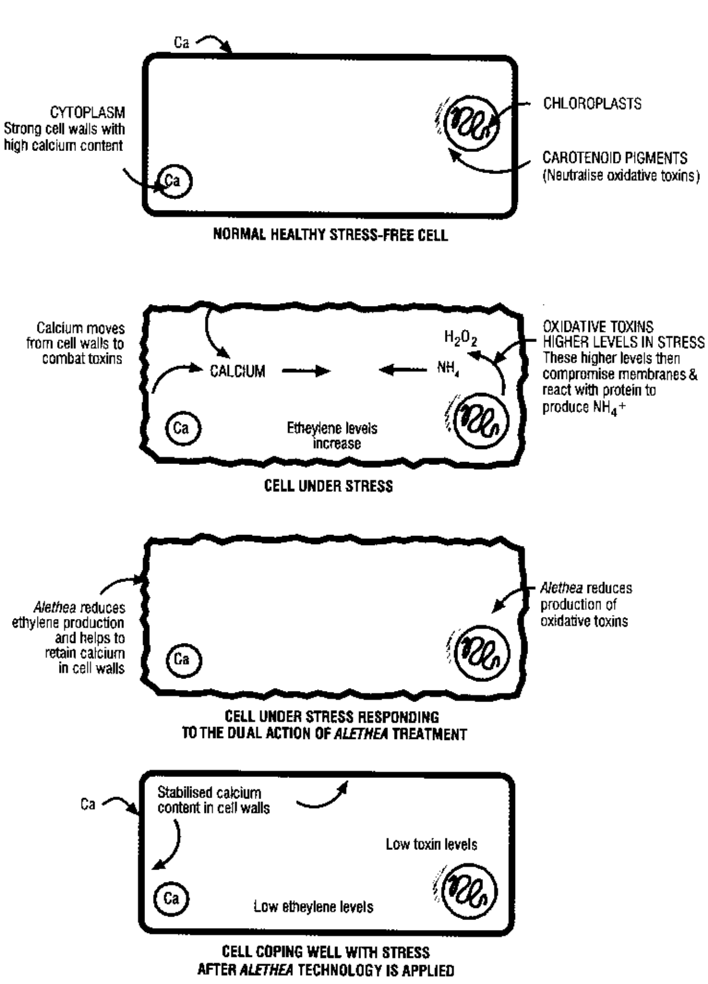
Two main mechanisms are best recognised, says Plant Impact's Marks. The first involves an escalation of the hormone ethylene, associated with fruit ripening. When plants are stressed, they produce high levels of ethylene which in turn promotes the migration of calcium from plant cell walls into the surrounding cytoplasm, where it is involved in neutralising damaging reactive oxygen species generated when plants come under attack.

Loss of calcium, however, is a double-edged sword, simultaneously weakening the plant and leaving it more vulnerable to infection and attack. As stress conditions are alleviated, therefore, plants respond by producing salicylates – chemicals similar in structure to the active ingredient acetylsalicylic acid in aspirin – that reduce ethylene production and promote the formation of polyamines that can substitute for calcium in the cell walls.

The second main mechanism for stress reduction is by increasing the production of antioxidants, including pigments called carotenoids that are associated with chloroplasts – the main source of reactive oxygen species.

'Because all kinds of stress affect all species of plants in fundamentally the same way – through damage by reactive oxygen species – effective stress-tolerance chemistry is likely to be effective across the full range of climates and crops,' says Marks.

The major problem with most earlier approaches to combat plant stress, however, has been lack of consistency, experts say. One of the few successful compounds to reach the marketplace is Syngenta's *Bion* (acibenzolar-S-methyl), which has been on the market since 1996 and wards off bacterial and fungal pathogens by mimicking salicylic acid. Interest in the agrochemical uses of salicylate chemistry dates back to the 1970s, and also stems from their ability to elicit production of natural 'plant antibodies' called phytoalexins as well as to reduce ethylene production. But efforts to harness these natural defensive properties have generally been disappointing, as besides improving plant strength, salicylates can also promote oxidative stress which may leave plants even more vulnerable to infection.




**Cells under fire: responding to stress**

> *'There is unlikely to be any one "silver bullet" capable of switiching on a stress tolerant trait for all types of plants'*

The trick with *Alethea* technology, according to Marks, is to combine a salicylate-type compound, benzoic acid, with a novel molecule called magnesium dihydrojasmonate. While the benzoic acid donor helps to strengthen the plant cell walls, the magnesium dihydrojasmonate accelerates the production of antioxidants needed to cope with the increased levels of reactive oxygen species.

Although still at the research stage, Marks acknowledges, *Alethea* is already included in a few commercial products now being tested in field trials on a range of crops including wheat, onions and potatoes in Saudi Arabia. The initial results should be available later this year.

While the early results with cocoa plants look encouraging, 'it would be interesting to see the results of field trials conducted over more than one season on a range of crops and a variety of different stressors to see how consistent the effect of *Alethea* is,' Straszewski says.

Meanwhile, Plant Impact is currently 'in evaluation agreements with several of the industry "big six" with *Alethea*,' Marks adds: 'So it seems that all of a sudden abiotic stress is topical in the industry.'