ANNUAL REPORT

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Project number:	PC 221
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Report:	Annual Report
Project consultants:	
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The results and conclusions in this report are based on a series of crop scale observations, crop trials and more detailed field- and laboratory-based experiments. The conditions under which the studies were carried out and the results have been reported with detail and accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with the interpretation of the results especially if they are used as the basis for commercial product recommendations.

Authentication

I declare that this work was done under my supervision according to the procedures described herein and that this report represents a true and accurate record of the results obtained.

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CP19 Horticultural crops: Further demonstration of the potential benefits of modified plastic crop covers report which forms 70% of the Fellowship is submitted separately.

2006 projects summary

Publications

Can transgenerational resistance to insect pests be exploited in commercial crop production?

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In response to attack by insect pests plants induce a wide range of defences for the purpose of minimising damage at the time of attack and reducing the likelihood of future attack. Because seeds of attacked plants develop in the maternal environment and that environment may predict the type of conditions that offspring will encounter, herbivory may have a delayed effect on plant fitness by acting to induce defences across generations of plants if the progeny of attacked plants are more resistant to attack than the offspring of undamaged plants. We report here that Tomato (*Lycopersicon esculentum* Mill cv. Carousel) repeatedly treated with Jasmonic acid (JA), a known chemical elicitor of induced resistance to herbivores, every three days from emergence of the first true leaf to harvest of the first ripe fruit increased resistance to *Tetranychus urticae Koch* **in the first generation progeny by $\geq$56% compared to controls. There was no effect of jasmonic acid treatment in the maternal generation on seed mass, time to emergence or vegetative biomass in progeny. These results demonstrate that the maternal environment determines, at least in part, the defensive phenotype of progeny. We discuss the underlying epigenetically inherited mechanism mediating such transgenerational responses and how this effect could be exploited through simple changes to current commercial seed production practices for the purpose of reducing the damage caused by economic pests and therefore the requirement for pesticides in commercial crop production.**

INTRODUCTION

Most plants are subject to parasitism by chewing insects and the associated damage can negatively impact plant fitness (Agrawal 2000; Moore et al 2003). For this reason herbivory may select for defences that act to reduce the frequency and scale of attack, or to reduce the growth and / or reproductive consequences for the plant (Agrawal 2002). Because plants as sessile organisms and have no way of avoiding injury caused by chewing insects or large herbivores they have evolved pre-existing physical barriers that act to minimise damage. These include the cuticle, which restricts herbivore grazing, or trichomes and thorns, which make access to certain plant parts difficult (Gomez & Zamora 2002; Karban & Baldwin 1997). However, if these barriers fail and the plant is injured, cells are capable of mounting a defence response through the transcriptional activation of specific genes (Leon, Rojo & Sanchez-Serrano 2001). The initiation of these responses act to direct the healing of damaged tissue and stimulates defence mechanisms for the purpose of minimising future damage (Leon, Rojo & Sanchez-Serrano 2001).

Depending on the species, and the type and level of damage caused, local defence responses may be activated within minutes, or perhaps hours, and include the generation, perception and transduction of signals leading to defence gene activation (for review see de Bruxelles & Roberts 2001). The proteins these genes encode act to inhibit herbivore performance by changing the digestibility of the tissue (Jongsma *et al*. 1995), or through toxin synthesis (Griffitts *et al*. 2001), but also mediate wound repair (Leon, Rojo & Sanchez-Serrano 2001), and play a role in altering plant metabolism (Broddmann *et al*. 2002). Such inductive responses can be adaptive in that their activation can increase the fitness of plants in the presence of herbivores (Agrawal 1998).

While herbivory leads to the activation of defence related changes it always, by necessity, leads to a reduction in leaf area and resources for the plant. Such reductions in the ability of the plant to fix resources can directly decrease growth, survivorship and reproductive success (Karban 1997). Therefore herbviory can directly influence plant fitness by affecting the number and / or vigour of the plants progeny. We propose here that herbivory may have a delayed effect on plant fitness by changing the behaviour of its progeny to insect pests. Seeds develop in the maternal environment and that environment may predict the type of conditions that offspring will encounter. Thus such transgenerational effects of herbivory may provide, through a so-far unidentified mechanism, an instrument for adaptive maternal changes in plant induced resistance.

In experiments carried out at Stockbridge Technology Centre we report an experimental investigation of such effects in a commercially grown tomato crop. We hypothesised that repeated activation of the pathways linked to defence against herbivores using a recognised chemical inducer of resistance, jasmonic acid, from germination through to fruit production would produce more resistant progeny. We discuss the possible underlying epigenetically inherited mechanism mediating such transgenerational responses and how this effect could be exploited, through simple changes to current commercial seed production practices, for the purpose of reducing the damage caused by economic pests and the requirement for pesticides in crop production.

MATERIALS AND METHODS

PLANT MATERIAL AND JASMONIC ACID TREATMENT

Seeds of Tomato (*Lycopersicon esculentum* Mill cv. Carousel) were sown in Levington no. 2 compost (Keith Singleton, Egremont, UK) in 58 cm^3 commercial blocks to germinate. At 35 days they were transferred individually into 160 mm plastic pots filled with Levington no. 2 compost before being split into two completely random groups of 15 plants each and were isolated (so pollination only occurred within treatment populations using hand pollination) in individual glasshouses at Stockbridge Technology Centre, North Yorks, at a temperature (26ºC day/ 13ºC night ± 3.0ºC) in natural light during June-July 2005. Leaves only of the Jasmonic Acid (Sigma, UK) treatment group were sprayed to dripping point with a solution of 3mM JA in 0.2% ethanol every 3 days until the first fruit had ripened and was then harvested. Controls were similarly treated with 0.2% ethanol only and the first fruit was similarly harvested. One seed was randomly chosen from each of the control and JA treated fruit. These were weighed and then germinated in isolated glasshouses as described above at a temperature (18ºC day/ 10ºC night ± 4.0ºC) in natural and supplemented light during October 2005 - January 2006. First generation progeny plants from both maternal Control and JA treatments were allowed to develop under identical conditions, with no treatment, until full expansion of the fourth leaf at 95 days post germination at which point *Tetranychus urticae* were applied.

APLICATION OF *TETRANYCHUS URTICAE*

Six adult female (2-3 days old) *T. urticae* were placed on the middle leaflet of the first fully expanded leaf of a tomato plant (*L. esculentum* Mill cv. Carousel). There were 10 plants per treatment. Each plant was grown in a 3L pot in a glasshouse (16L: 8D, min^{m} temp. 15^{o}C, venting at 24 ^{o}C). After seven days the plants were removed and the numbers of live adult and offspring were recorded for each treatment.

RESULTS

There was no effect of maternal treatments on seed weight (P>0.05, Kruskal-Wallis one way ANOVA; Fig 1.a) or time to emergence in first generation JA treatments (11 ± 2.96) when compared to Controls (10 ± 1.47) (P>0.05, Kruskal-Wallis one way ANOVA; data not presented).

Repeated application of JA to maternal plants resulted in a 41% reduction in the number of live adults after 9 days on first generation progeny when compared to first generation Controls (P<0.05, Students t-test, Fig 1.b). The total number of eggs produced was also reduced by 56% in JA treatments (P<0.001, Students t –test; Fig. 1.c) and although there was a 35% reduction in the total number of eggs produced per live adult at the end of 9 days this did not represent a significant reduction (P>0.05, Students t-test, Fig. 1.d).

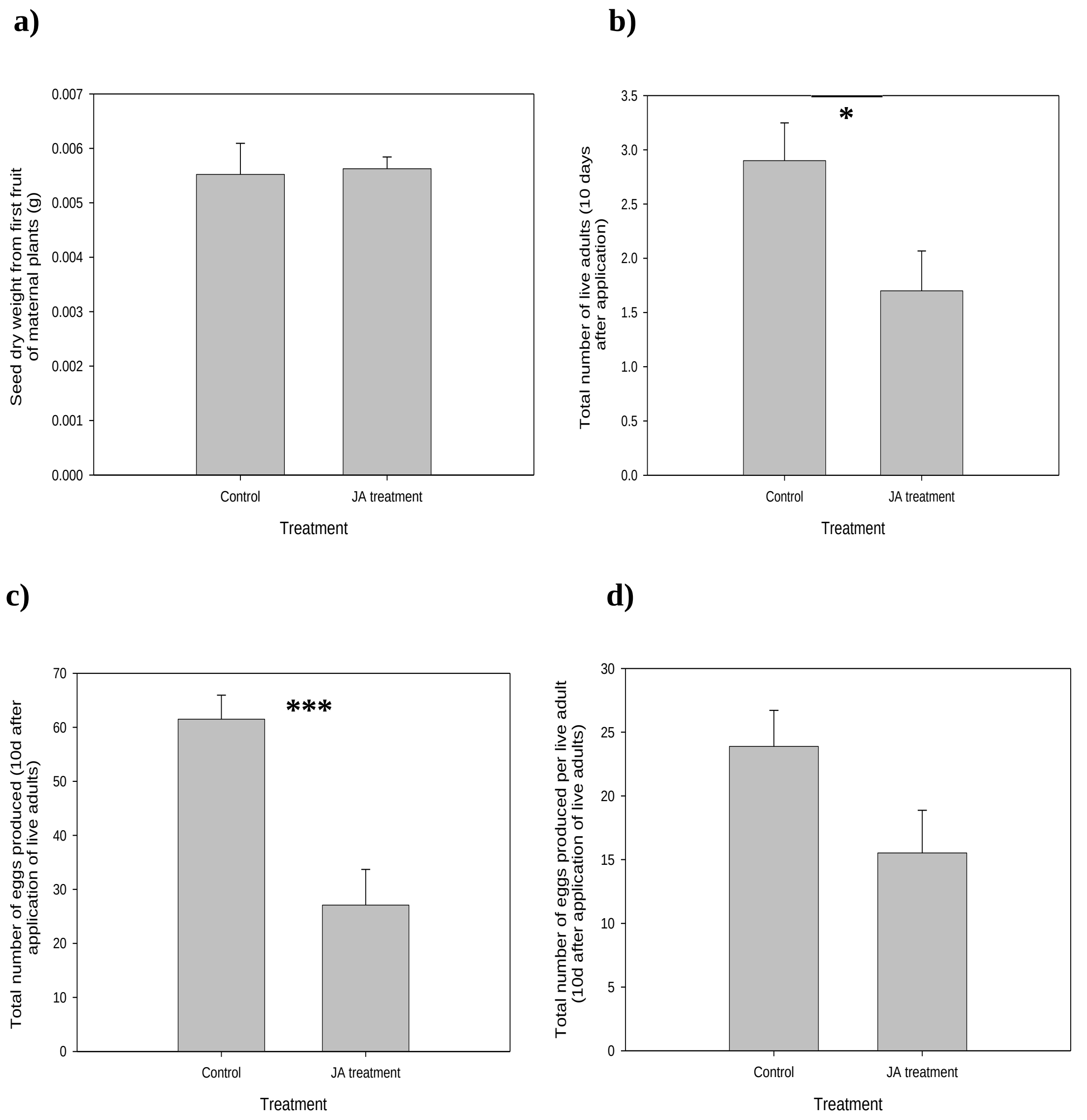

Figure 1. (a) Seed dry weight in first fruit of maternal plants, (b) the number of live adult mites on (*Tetranychus urticae)* per unit area of leaf four of first generation progeny, (c) total egg production and (d) total number of eggs produced per live *Tetranychus urticae* on first generation progeny plants of JA treated and Control tomato cv. Carousel. n=10 + s.e. *P<0.05, ***P<0.001.

DISCUSSION AND FUTURE WORK

The repeated induction of resistance in maternal plants had an effect on the resistance of first generation progeny (Figs. 1.b. -1.d). Repeated treatment of maternal plants with jasmonic acid (JA) decreased *Tetranychus urticae* survival by 41% and the number of eggs produced by 56% compared to controls. There was no effect of treatments on progeny seed mass (fig. 1.a) or the time to emergence (data not presented).

These results indicate that repeated priming of the pathway(s) linked to herbivore resistance in *Lycopersicon esculentum*, using exogenously applied JA, causes maternally induced resistance. In this study maternal plants were treated with JA from emergence through to the time when the first fruit had ripened, although fruits were isolated at the time of spraying and only foliar tissue was targeted. Thus the maternally inducing signal must be translocated from the vegetative tissue, through the developing fruit to the seed. In order to explain the increased levels of first generation progeny resistance reported here this signal must then act to alter the behaviour of the embryo through development into early maturity (>95 days) as a first generation hereditary trait.

For three decades, largely due to the work of Richard Dawkins, it has been widely accepted that the fundamental unit of selection is the gene, the unit of heredity (Dixon & Dawkins 1988). This hypothesis has so dominated discussion that other levels of selection or forms of inheritance has been largely ignored. However, recent studies from both the animal and plant kingdoms suggest that perhaps too much emphasis has been put on the gene-centred approach and that there are forces acting on evolution other than orthodox genetics (Jablonka & Lamb 1998; Regev et al 1998). There is now growing evidence that non-genetic information affecting development is routinely passed from one generation to the next (Jablonka 2003). This developing paradigm suggests that certain chemical ‘methyl’ groups “hitchhike” on genes leading to different interpretations of that gene. This heritable, non-genetic “hitchhiking” is known as epigenetic inheritance. There are many examples of heritable changes transmitted by something other than genes and in a landmark study Anway et al (2005) reported that initial exposure to two pesticides reduced sperm counts in at least the subsequent four generations of male rats. The effect did not seem to be the result of changes in the DNA sequence, making it the first time any chemical has been shown to cause any heritable effect other than by random mutation (Anway et al 2005).

There is also increasing evidence also from plant (Katop et al 2004; Agrawal 2002), bird (Naguib et al 2005) and insect (Mondor et al 2005; Podjasek 2005) studies that transgenerational effects act through epigenetically inherited changes in gene expression interpretation to influence offspring behaviour. In humans also the transgenerational effects of maternal nutrition or other environmental 'exposures' in human populations are becoming recognised (Khan et al 2005) and, perhaps surprisingly, recent work has indicated the possibility that exposure to certain compounds in men influences development and health in the next generation male (Pembrey et al 2005). They concluded that sex-specific, male-line transgenerational responses exist in humans and hypothesise that these transmissions are mediated by the sex chromosomes, X and Y.

Possible mechanisms of transgenerational resistance in plants

In plants, there is now initial evidence that newly acquired epigenetic states of transcriptional gene activity are transmitted to progeny (Takeda & Paszkowski 2006). This transgenerational inheritance of new epigenetic traits seems to rely on cytosine methylation maintained through meiosis and postmeiotic mitoses, giving rise to gametophytes (Takeda & Paszkowski 2006). DNA methylation in eukaryotic cells involves the addition of a methyl group to the carbon at position 5 of the cytosine ring. This reaction is catalyzed by the enzyme DNA methyltransferase (DNA-MTase) and this methylation reaction is the most common covalent modification occurring in eukaryotic DNA (Takeda & Paszkowski 2006).

Whether, and at what level, methylation acts to produce transgenerational changes in insect resistance in higher plants is not clear but one possibility is that it alters the synthesis of and / or the sensitivity to key molecules that regulate defence, possibly through increased production of receptors to those molecules. One such key signalling molecule linked to plant responses to pest resistance in all higher plants is jasmonic acid. JA is synthesised through the octadecanoid pathway and is a key regulator in the physiology, development and defence of plants with the complexity of this signalling pathway only just emerging (Schaller 2001; Schenk *et al*. 2000). Accumulation of JA is observed at high levels in damaged tissue and increases are also observed in systemic leaves making JA a leading candidate as the primary component of systemic, whole-plant responses to herbivory (Rojo *et al*. 1999; Laudert & Weiler, 1998). In this study repeated exogenous application of synthetic JA was applied to the maternal treatment group and this positively altered the behaviour of first generational offspring to insect attack. If correct and methylation driven transgenerational changes in key defence related signalling pathways is utilised by plants for the purpose of increasing phenotypic plasticity in response to insect pest related stress it opens up the possibility to alter progeny behaviour to other important economic stresses: including disease.

Plant responses to pathogens and possibilities for transgenerational disease resistance?

The primary localised disease defence response is often referred to as the hypersensitive response (HR). The HR is characterised by rapid, local death of plant cells at the sites of pathogen infection and is a common feature of non-compatible plant-pathogen interactions (Kumudini, Vasanthi & Shetty 2001). This programmed cell death (PCD) of affected tissue restricts the spread of pathogens from the infection site and is considered one of the most effective plant resistance mechanisms, since it is highly effective in limiting pathogen spread (Jones 2001; Lam, Kato & Lawton 2001). The HR is accompanied in many instances by the generation of locally synthesised signalling molecules including reactive oxygen species (ROS), such as superoxide (O_2^-), hydrogen peroxide (H_2O_2) (Bolwell 1999; Bolwell & Wojtaszek 1997) and NO (Delledonne et al 2002; Durner & Klessig 1999), which occurs before and during lesion-associated host cell death (de Pinto, Tommasi & de Gara 2002; Pellinen *et al*. 2002). The simultaneous increase of NO and ROS in tobacco has been reported to activate a process of death with the typical cytological and biochemical features of hypersensitive PCD and a rise in phenylalanine ammonia-lyase (PAL) activity (de Pinto, Tommasi & de Gara 2002). PAL is involved in salicylic acid formation (Métraux 2002; Mauch-Mani & Slusarenko 1996), the accumulation of which is implicated in many cases with the subsequent systemic expression of a large number of defence-related genes, leading to the development of systemic acquired resistance (Morris *et al*. 1998).

Systemic acquired resistance (SAR) is the fundamental defence mechanism induced by a wide range of pathogens (Cordelier *et al*. 2003; He, Hsiang & Wolyn 2002; Hennin, Diederichsen & Hofte 2002; Ryals *et al*. 1996). The induction of SAR leads to the expression of genes and production of numerous proteins in several plant species including maize (Morris *et al*. 1998), tobacco (Song & Goodman 2002), *Arabidopsis thaliana* (Dong, Chen & Chen 2003; van der Biezen *et al*. 2002), soybean (He *et al*. 2001), and pepper (Lee, Kim & Hwang 2002), although their nature and the level at which they are expressed various among species. Increases in SAR gene expression and mechanical changes in the host cell wall are thought to be responsible for increased resistance in non-infected, secondary plant tissue and pathogen induced SAR in many plants is often preceded by increases in levels of SA.

In a similar manner that exogenously applied JA induces insect pest resistance exogenous SA treatment in tobacco and other plant species activates a diverse spectrum of (*PR*) proteins leading to resistance to pathogens (Kessmann *et al*. 1994). Induction of disease resistance can also be achieved by spray treatments with synthetic mimics of SA such as isonicotinic acid and benthiadiazole (Durner, Shah & Klessig 1997) and for this reason we hypothesise that one, or all of these compounds, could be used to epigenetically alter progeny behaviour in response to economically important pathogens.

Future possibilities

The body of evidence for transgenerational changes in plant behaviour through epigenetic inheritance is accumulating as it becomes increasingly evident that the gene-centred approach does not possess the explanatory power to account for the variety and complexity of organisms. Because epigenetic inheritance would appear to act very quickly, within the first generation offspring and in response to limited stress signalling (results reported here and Agrawal 2002) and in response to exposure to certain biologically active compounds (Anway et al 2005) this opens up the possibility of altering plant responses to biotic stresses in a straightforward and both economically and environmentally beneficial manner.

It is widely accepted that commercially produced crops are more susceptible to insect pests than wild populations and results reported here raise the possibility that this could be linked to the lack of biotic stress in growing conditions of plants used for seed production. In a conventional system maternal plants are protected from pests using conventional pesticides in the belief that favourable growing conditions will produce the most productive progeny in terms of harvestable yield. However, if maternally induced resistance to insect pests is a wide-spread phenomenon throughout the plant kingdom and plants grown for seed are protected from pests, then seed being produced for commercial crop production may be particularly susceptible. This susceptibility could be overcome by changing commercial seed production practice in economically neutral ways by substituting traditional pesticides for known inducers of both pest and disease resistance. This approach would induce high levels of pest resistance in maternal plants meaning that leaf and therefore resource driven yield loss would be minimised in the first instance and in the second, positive epigenetically driven defence changes would be expressed in progeny. In conclusion, as well as providing an entirely new dimension to the study of gene–environment interactions, such transgenerational epigenetic driven effects in crop plants may provide a tool for altering crop behaviour in response to economically important biotic stresses without the requirement for genetic modification through minimal changes in commercial seed production techniques.

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Increasing postharvest resistance in protected crops by external application of trans-Resveratrol.

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The problem of how much crop is lost after harvest to processing, spoilage, insects and disease, or to other factors, takes on greater importance as world food demand grows. Cutting postharvest losses could, presumably, add a sizable quantity to the global food supply chain. Traditional solutions to this problem such as storage under controlled conditions and the use of synthetic pesticides are not risk free due to the toxic nature of the chemicals used. An alternative solution to extending the useful life of food crops consists of exploiting the crops own natural resistance strategies in suppressing pathogen colonisation utilising plant derived compounds. Trans-resveratrol is a naturally occurring phytoalexin produced by some higher plants in response to injury or fungal infection leading to resistance to these pathogens. In this preliminary study organic apples were exogenously treated with trans-resveratrol and their postharvest quality was visually monitored over a period of 35 days in order to determine the compounds efficacy in extending commercially relevant shelf-life.

INTRODUCTION

Fresh fruits and vegetables are inherently perishable. During the process of distribution and marketing substantial losses are incurred: ranging from a slight loss of quality to total spoilage due to pathogen attack and natural senescence. Losses vary greatly by crop, by country, and by climatic region, and partly because there is no universally applied method of measuring losses little solid information exists on the precise amount and nature of wastage worldwide (Mazaud 1997). As a consequence, estimates of total global postharvest food loss are controversial and range widely—generally from about 10 percent to as high as 40 percent in some studies (Satin 1997).

Traditional solutions to this problem such as storage under controlled conditions and the use of synthetic pesticides have inherent environmental and human health issues due to the nature of the chemicals used and so alternative methods have been sought. One novel strategy for alleviating the problems associated with the use of synthetic pesticides is the development of methods to improve the crops natural resistance to postharvest pathogens using the plants own secondary metabolites. In recent years considerable effort has been directed towards identifying such functional pest suppression metabolites and understanding host-parasite interactions in the postharvest period (Baket et al. 1997, Keen 2000, Kutchan 2001). Although in-roads have been made in terms of understanding these complexities and this has led to the subsequent development of first generation disease-resistant transgenic plants it is highly unlikely that the underpinning technology will be available to UK growers in the near future (Broglie et al. 1991, Martin et al 2003, Adrian et al 2000). For this reason attention is now turning to inducing the plants own defences, or employing plant derived defence or antifungal compounds, for the purpose slowing post harvest deterioration.

Resveratrol (3,4',5-trihydroxystilbene) a natural compound found in many dietary plants and in red wine, plays an important role in the prevention of a number of human pathological processes, including inflammation (Kimura 1985), atherosclerosis and carcinogenesis (Pace-Asciak et al 1995; Mgbonyebi et al 1998). This phenolic antioxidant has been identified as a potential cancer chemopreventative agent (Jang et al 1997), its presence in red wine has been suggested to be linked to the low incidence of heart disease in some regions of France (Renaud & DeLorgeril 1992) and recent studies have shown that it acts to modulate the activity of the *SIR2* and *SIRT1* genes and its siblings (collectively referred to as Sirtuins) which have been directly linked to increased longevity in yeast and certain mammals respectively (Howitz et al 2003; Sinclair 2005). Phytoalexins, including resveratrol, have also been shown to be important natural components in the defence of plants against fungal infection (Bernard et al 1998). Under a pathogen attack, plants have evolved sophisticated systems of detection and response to decipher the pathogen signals and to induce appropriate defences. These systems include specific networks that operate through the action of signalling pathways mediated by molecules such as salicylate, jasmonate, and ethylene and generate the accumulation of pathogenicity-related proteins, phytoalexins, or other phenolic compounds (Elad, 1997; Dong, 1998; Feys and Parker, 2000). A recent study showed that trans-resveratrol is fungitoxic at physiological concentrations against *B. cinerea* (Adrian et al., 1998) and in-vitro production and exogenous application of trans-resveratrol has also proven to enhance the resistance of vineplants to other pathogens, such as *Plasmopara viticola* (Dai et al., 1995), *Phomopsis viticola* (Hoos and Blaich, 1990), or *Rhizopus stonifer* (Sarig et al., 1997). This rather unspecific antifungal character and the selective accumulation of trans-resveratrol in grape skin has made it a good candidate as a natural pesticide against pathogen attack for improving the natural resistance of, both prior and post harvest crops, against a diverse range of economically important pathogens.

MATERIALS & METHODS

Organic apples (Pink lady) were purchased and briefly washed in warm water to remove impurities. The fruits were then split randomly into treated and non-treated groups. The treatment group were dipped in a 1.6×10^{-4} solution of resveratrol (Sigma, UK) in deionised water for a period of one minute. The non-treated group were dipped in deionised water alone for the same period. Following treatment fruits from both groups were kept in the open air at a day / night temperature 21°C – 19°C in a well lit room but out of direct sunlight. A visual record of fruit quality was then kept for a period of 35 days.

RESULTS

Figures 1 and 2 clearly show the effect of trans-Resveratrol treatment at days 0, 7, 14, 21 and 35 on visual quality of organic apples (Pink lady) when compared to untreated controls. The treated fruit clearly maintained a nondeteriorated aspect with little external sign of decay up until day 21, while the non-treated fruit exhibits early deterioration by day 7 and is rotten and of no value by day 35.

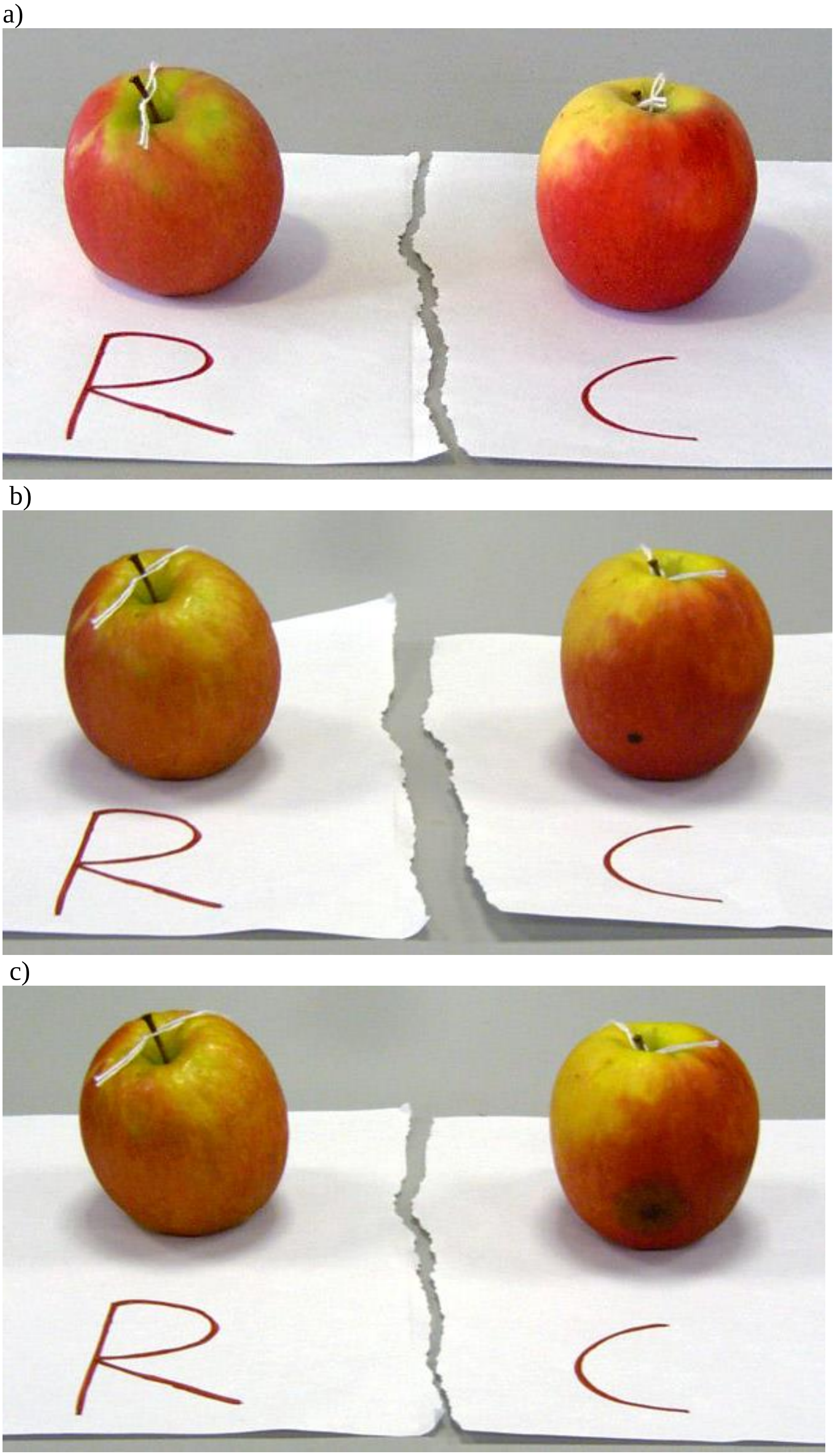

Figure 1. Effect of Resveratrol (R) treatment on visual quality of organic apples (Pink lady) when compared to control (C) at day a) 0, b) 7 and c) 14.

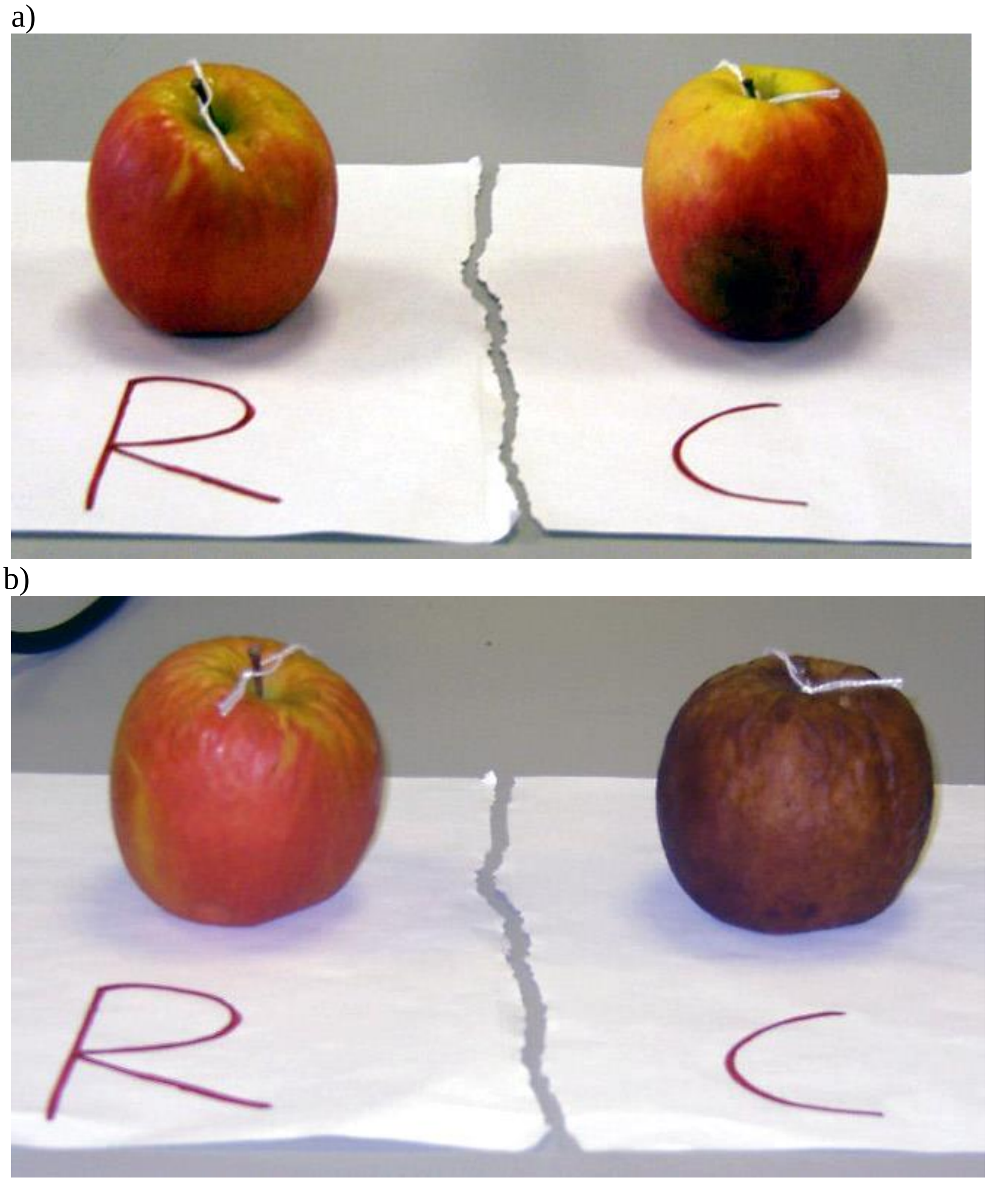

Figure 2. Effect of Resveratrol (R) treatment on visual quality of organic apples (Pink lady) when compared to control (C) at days a) 21 and b) 35.

DISCUSSION

The organic apples were immersed into a solution containing 1.6×10^{-4} m trans-resveratrol for one minute, as indicated in “Materials and Methods.” As shown in Figures 1 and 2, significant differences between the untreated apples and those treated with trans-resveratrol were obtained 35 d after treatment. The trans-resveratrol treated fruit clearly still maintained a physical aspect with little sign of losses or deterioration, whereas the untreated fruits were not only dehydrated, but exhibited infection and deterioration, showing local development of fungi, as one would expect after this period of time because normal shelf-life of apples at room temperature is about 5 days. Although the antifungal character of trans-resveratrol has been widely reported, mainly by in vitro investigations in grape, these preliminary results indicate that direct exogenous application of resevratrol to apple acts to prevent fruit deterioration through a so far unknown mechanism(s).

There are multiple interacting components of crop deterioration including the development of various yeasts and fungal pathogens such as *B. cinerea*, *Penicillium*, *Aspergillus* and *Alternaria* spp. The ability of certain fruit crops, including many red grape varieties, to resist pathogen infection throughout development to harvest has been linked to the in vitro production of a family of phytoalexins produced by some higher plants in response to injury or fungal infection. Among these reactions are the phytoalexins, low molecular mass products of the secondary plant metabolic pathway. They are often synthesised within hours after stress exposure, accumulate and reach a maximum 2-3 days after induction (Purkayashita 1995). The family of phytoalexins which have been well characterised constitute a rather restricted group of molecules belonging to the stilbene family, of which resveratrol is a member (Jeandet et al 2002). Resveratrol is the major phytoalexin produced in grapevine as a general resistance response to fungal attack (Langcake & Pryce 1976), leaf UV-C irradiation (Bouquet et al 1982), heavy metal exposure (Adrian 1996) and in response to ozone treatment (Schubert et al 1997), which strongly indicates that it acts as an effective, non-specific anti-stress compound. Furthermore, resveratrol acts directly on both the *Sir2* and *SIRT1* genes (members of the conserved sirtuin family of NAD(+)-dependent protein deacetylases) and changes in the activity of these genes are known to lead to increased DNA stability and extended lifespan in a diverse range of organisms; from yeast to certain higher mammals (Sinclair 2005). Given that resveratrol is manufactured in plants that are stressed and at least 18 other compounds produced by plants in response to stress have also been found to modulate Sirtuins (see Lammin et al 2004) it is not unreasonable to suggest that plants may use such molecules to control their own *Sir2* enzymes for the purpose of extending lifespan. Therefore targeting *Sir2* activity using one or more of these 18 so far identified compounds may provide a good starting point for the large scale screening of active molecules for extending crop shelf life.

One of the main stages in the development of new natural pesticides is the study of the toxicological and environmental properties of the compound to be used and plant derived biological control compounds are one of the more interesting alternatives to the use of traditional chemical pesticides (Duke, 1990). While a number of reports have suggested that the risks for human health related to the consumption of natural chemicals in food crops can be even greater than those from pesticide residues (Pimentel et al., 1996; Swirsky et al., 1997), the lack of toxicity of resveratrol has already been demonstrated. Indeed, in the case of resveratrol, a considerable number of investigations are currently focussed on the health benefits of resveratrol consumption (for recent reviews on this subject, see Frémont, 2000; German and Walzem, 2000; Parr and Bolwell, 2000). There is little doubt that the problem of food loss is globally significant and therefore the challenge over the coming years will be to determine just how much food (and economically important non-food crops e.g. cut flowers) loss can be prevented by employing plants own secondary metabolites to combat postharvest deterioration.

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Humates and their relevance in commercial crop production: a short review.

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Summary

The use of commercial humates in UK crop production has been received with mixed reactions. Wide variations in the origins of commercial humates and soil humic acids coupled with a lack of scientific data on the former are contributing to the controversy. Humic substances are complex, naturally occurring organic compounds of high molecular weight, participate actively in the decomposition of rocks and minerals and promote the conversion of a number of elements into forms available to crop plants. The commercial literature makes varied claims as to the active benefits of humates on crop yield and quality but the scientific literature reports more varied, sometimes contradictory, responses. The lack of uniform responses may be linked to the structural characteristics of the varying products used which in turn are dependent on the origin of the original matrix. For this reason the general roles of humates in soil ecosystems have been extensively studied but uncertainty remains over the mechanism(s) by which these substances may influence the biological activities of microorganisms and plants. This short paper reviews the current literature on humates with specific reference to the current body of evidence related to crop responses to these substances and the proposed direct and indirect regulatory mechanisms mediating these responses.

Background to humates and humic substances

The term *humic substances* refers to a loosely defined, heterogenous mixture of naturally occurring organic materials; are ubiquitous in nature and are formed from the decay of both animal and plant residues in the environment. Humic substances, the major component of soil organic matter are often classified into three broad categories: humic acid (the fraction of humic substances that is not soluble in water under acidic conditions (pH<2) but is soluble at higher pH values), fulvic acid (the fraction of humic substances that is soluble in water under all pH conditions) and humin (the fraction of humic substances that is not soluble in water at any pH value) (Chen and Aviad 1990). A number of indirect, nutrient driven beneficial crop related effects of humates and its components have been characterised in the literature. However, others, such as the proposed direct effect on crop performance through changes in chemical signalling and plant metabolism and the active mechanisms regulating these responses are still largely controversial. The extreme heterogeneity of humates is regarded as one of their inherent properties but the underlying mechanisms driving possible physiological and the gross crop effects of these substances will remain obscure until their specific nature is quantified (Vaughan and Malcolm 1985).

It is known that humic matter is composed of aromatic rings that interact with aliphatic chains producing macromolecules with different masses (Chen and Aviad 1990). However, the genesis of humic substances involves numerous reaction pathways and a wide spectrum of binding systems which makes defining their exact composition problematic (Hayes 1997). Further complications have arisen due to recent research which has questioned the validity of the ‘high molecular weight’ component of humates (Canellas et al. 2000). These ‘high molecular weight’ non-humic substances are discrete compounds which can be categorized (e.g. polysaccharides, amino acids etc) and are readily degraded by micro-organisms, unlike humic substances which are broken down much more slowly (Canellas et al. 2000). Because there is no clearly defined cut-off between these fractions and they can be considered to be a continuum research focuses predominantly on defining the broad characteristics of these substances and attempts to understand their effect on the structure and performance of soil. The inherent problem with defining specific mechanistic effects of humic fractions on soil and plant performance is the sheer range and complexity of the defining components of humic substances and the associated problems with separating them into pure components.

Virtually every separation technique that has been developed by chemists and biochemists has been applied to these humic substances and The International Humic Substances Society (IHSS) has established standard procedures for isolating the aforementioned fractions. Examples of techniques employed include acid hydrolysis, oxidative degradation, amalgam reductive degradation, pyrolysis-mass spectrometry, nuclear magnetic resonance spectrometry and electron spin resonance spectrometry (Abe et al. 2005; Schnitzer and Ortiz de Serra 1973; Kujawinski 2002; Hervas et al. 1989). It has been found that C, H, O, N, P and S generally account for 100% of the composition of humic substances on an ash-free basis (Allard 2006). The major functional groups in humic substances are carboxyl, alchol, phenolic hydroxyl and carbonyl (Hernandez et al. 1988) with lignins and fungal melanins believed to be a key source of the aromatic units in soil humic acids (Göbbels and Püttmann 1997). Their structures are partially degraded, transformed, and recombined through enzymatic and autooxidative reactions to form stable humic macromolecules (Gossart 2003). The transformation of these molecules (humification) is the natural process of changing organic matter, such as leaves, into humic substances by geo-microbiological mechanisms with compost being the intermediate product consisting of humic substances and partially decomposed organic matter. As the conversion process continues, different chemicals dominate at different points in time (Ziechmann, et al. 2000). Complete conversion to humic substances will eventually occur but with the infinite variety of both plant materials and access to chemical radicals, humification should produce humic substances that are immeasurably variable and therefore possess infinite effects on soil ecosystems and crop performance (see table 1 below) (Ziechmann et al, 2000).

Almost since the inception of farming the benefits of soil organic matter on crop productivity have been recognised. While uncertainty surrounds many of the supposed specific effects of humates there is some general evidence that they 1) aid both plant and microbial growth by providing a source of N, P and S essential for nutrition, 2) they act to moderate soil pH changes, 3) provide a mechanism for maintaining soil moisture content, 4) binds essential micronutrient metal ions into the soil, 5) protects against soil erosion by binding the constituent parts of the soil together while its dark colour alters albedo allowing the absorption of solar energy and heating the soil (Nardi et al 2002; Ertli et al 2004; Galantini and Rosell 1997). However, because soil is inherently complex, consisting of multiple, interacting organic and non-organic components ascribing specific roles in terms of both direct and indirect effects on crop productivity is a matter of continuing debate in the scientific literature.

TABLE 1

Major classes of compounds and biomacromolecules identified in humic substances (adapted from Saiz-Jimenez, 1996):

Class of compounds	Possible origin
Aliphatic hydrocarbons	
Alkanes	Microbial/plant/pollutant
Alkanols	Microbial/plant/pollutant
Aliphatic acids	
Fatty Acids	Microbial/plant/pollutant
Hydroxy Fatty Acids	Microbial/plant/pollutant
Dicarboxylic Acids	Microbial/plant/pollutant
Alkylaromatics	
Alkylbenzenes	Pollutant/artefact
Alkylnapthalenes	Pollutant/artefact
Alkylphenols	Microbial/plant
Dialkyl phthalates	Pollutant
Aromatic hydrocarbons	
PAH	Pollutant
Aromatic acids	
Benzenecarboxylic acids	Microbial/plant
Phenolic acids	Microbial/plant
Other hydrocarbons	
Tocopherols	Microbial/plant
Chlorophylls	Microbial/plant
Terpenoids	Microbial/plant
Steroids	Microbial/plant
Biomacromolecules	
Waxes	Plant
Polysaccharides	Microbial/plant
Proteins	Microbial/plant
Lignins	Plant
Aliphatic macromolecules	
Sporopollenin	Microbial/plant
Algaenan	Microbial/plant
Cutan	Microbial/plant
Suberan	Microbial/plant

Effects of humates on plant biochemistry productivity

Humic substances are the subject of investigation in various areas of horticulture and agriculture, such as soil chemistry, fertility, plant physiology as well as many of the environmental sciences because of the wide variety of roles these compounds are purported to play in plant development (Tan 1998). It is widely accepted that soil fertility and plant growth are determined by mineral nutrition, as well as environmental and soil conditions and humankind has realized for thousands of years that dark-coloured soils with high humus content are more fertile than light-coloured soils (Scharpf 1967). Anywhere on the globe where there is soil or water associated with organic matter, humic substances are present. They are the brownish tint often seen in natural streams, the darkness of dark soils and the dark brown colour of weathered lignite coal. Humic substances are the most widely distributed organic products of biosynthesis on the face of the earth (Tan 2003), exceeding the amount of carbon contained in all living organisms by approximately one order of magnitude (Steinberg 2003).

The evidence for humate efficacy in field trials

It has long been reported that humic substances have many beneficial effects on soils and consequently on plant growth (Mulller-Wegener 1988). The economically important and beneficial impacts of humic substances on crop development at various physiological stages have been reported (Myloans & McCants 1980; Tan & Nopamornbodi 1979). In replicated field experiments carried out on tomatoes, peppers and strawberries using humic acid containing vermicomposts yields were reported to be increased (Arancon et al. 2003). Marketable yields were significantly increased, along with total leaf areas and the authors proposed that this was at least partially due to large increases in soil microbial biomass after vermicompost applications, leading to production of hormones or humates in the vermicomposts acting as plant growth regulators independent of nutrient supply (Arancon et al. 2003). In a study using lignite extracted humic acids on sugracane Govindasmy and Chandrasekaran (2003) reported a non-time dependent, significant increase in cane yield compared to controls. The addition of HA also improved the sugar yield as well as the concentration of nutrients in the leaf blades and sheaths (Govindasmy and Chandrasekaran 2003). In replicated field trials the addition of leonardite derived humic substances on tomato yield increased average yield by 10.5% related to untreated controls, by 11.29% in cotton and between 3-70% in vineyards (Brownell et al 2003). In long-term field trials using humic acid containing lignite fly ash (LFA) in rice crops reported both yield increase and improvements in the texture and crop fertility (Ram et al. 2005). In general the reported developmental and morphological effects of these substances are more apparent in root, rather than shoot, development and this could be related to direct effect (improvement of overall root biomass or architecture) and / or indirect effects (increase of fertiliser efficiency or reducing soil compaction) on plant biochemistry or nutrient uptake respectively (Vaughan and Malcom, 1985).

Direct mechanisms of humates in crop plants

In order for humic substances to act directly on plant metabolism implies that these compounds can cross the root boundary and possibly be translocated to distal plant tissue. The earliest work on humate uptake relied on colour changes in plant tissue, following treatment with humates, as an indicator of uptake (Prat 1963). More recent work has involved labelled carbon isotopes (Vaughan & Ord 1981) which showed that the amount of root associated radioactivity increased in conjunction with increases of both humic and fulvic acid (Vaughan and Ord 1981). Experiments using pea roots incubated in labelled humus at various temperatures suggested two uptake components: the primary was a rapid passive process while the secondary mode was slower but continuous active uptake dependent on plant metabolism (Vaughan and Ord 1981). Among the different modes by which it has been suggested humic substances influence biota, their surfactant like properties are the most widely reported (Nardi et al. 1991). Indeed the augmentative effect of humic compounds on biological membrane permeability has repeatedly been claimed as responsible for both better plant nutrition (Samson & Visser 1989), mostly through a higher ion uptake in root tissues (Varanini et al 1993). These functional aspects of the interactions of humic acids with crop plants are still far from certain but despite this various modes of action through which humic substances promote plant growth directly have been proposed.

Improved ion uptake

The effect of soil humus on ion uptake, and on general plant growth has been widely investigated (Vaughan and Malcom 19865; Chen and Aviad 1990; Varanini and Pinton 2001 and Clapp et al 2001). The results of these investigations suggest that ion uptake is highly variable and may be dependent on their concentration and the pH of the medium in which they are found (Vaughan and Malcom 19865; Chen and Aviad 1990). In beetroot disks the addition of humic acid increased the uptake capacity of Na+, Ba2+ (Vaughan and McDonald 1976) and phosphate (Vaughan and McDonald 1971). The increased capacity for Na uptake was related to increased protein synthesis; cycloheximide and D-threo-chloramphenical (both protein synthesis inhibitors) inhibited it (Maggiona et al 1987). Humic acid was not able to overcome this inhibitory effect and did not affect incorporation and distribution of ^{14}C-labeled amino acids into proteins (Vaughan and MacDonald 1976). Furthermore Albuzio et al (1986) reported that both high and low molecular sized humate fractions and humic and fulvic acid (Maggiona et al 1987) may affect NO_3, SO_4 and K+ uptake.

A study by Panuccio et al (2001) of nitrogen uptake in two different coniferous species; *Pinus pinaster* and *Pinus laricio*, reported that humic fractions at various concentrations influenced ammonium uptake whilst nitrate uptake remained largely unaffected. Seedlings of the two species were grown for 24h in hydroponic cultures containing a pre-prepared aerated humic fraction at various concentrations and spectrometric and colorimetric methods were used to assess the uptake / accumulation of both nitrate and ammonium. The addition of KNO3 to the culture stimulated nitrate uptake for both species. Low concentrations of the humic fraction also stimulated nitrate uptake in *P. pinaster* (Panuccio et al. 2001). However, high concentrations of the fraction resulted in inhibition of nitrate uptake in both species while ammonium uptake was increased with the presence of the humic fraction at both low and high concentrations in both species (Panuccio et al. 2001).

The effects of humic substances on ion absorption are not easily explained probably due to the complex and still largely unknown nature of these substances. The situation is further complicated because comparisons are difficult due to the origin of the soil used, the method of extraction used in different investigations and the fact that humic substances may effect several different plant functions modulating ion uptake simultaneously.

Humic substances act on membrane H^+-ATPase enzymes that enhance nitrate uptake

Primary active transport by plant cells depends on the presence of a vanadate-sensitive proton-pumping ATPase (H^+-ATPase) which builds up an electrochemical proton gradient across the plasma membrane (Morsomme and Bountry 2000). The latter energizes secondary active transport accomplished by carrier proteins via symport or antiport (Morsomme and Bountry 2000). In this context, NO3- is taken up by an inducible H^+ / NO3- symport (Miller and Smith 1996).

In experiments using Maize Pinton et al (1999) exposed 4 day old root seedlings from between 4 to 24h to a 200 µm nitrate supplemented with or without 5mg l^{-1} of a water extractable humic substances fraction. They reported a net uptake of nitrate by the treated seedlings which progressively increased up to 12h in the presence of nitrate alone. When the water-extractable humic fraction was added, net nitrate uptake was maximised after 4h and declined only slightly over a 24h period (Pinton et al 1999). The authors also reported that in conjunction with increased net nitrate uptake plasma membrane H+-ATPase activity increased when the water extractable humic fraction was in the medium after 4h. These results suggest that proton pumps in the plasma membrane may be one target of humic substances. Enhanced plasma membrane activity H^+-ATPase in response to humic acid has also been reported by Cannelas et al (2002). In this study, 40 mg l-q humic acids extracted from cattle manure earthworm compost were used to treat maize roots for 7 days. Plasma membrane vesicles from the roots exhibited a significant increase in vanadate-sensitive ATPase activity as well as of the formation of an ATP dependent proton gradient (measured as a quenching of ACMA fluorescence). This suggests that humic acid may affect H^+-ATPase activity indirectly by promoting an increase in the concentration of H^+-ATPase in the membrane vesicles.

Improved micronutrient, not N, uptake?

Micronutrient uptake by wheat and alfalfa plants in different soil types supplemented with humic extracts was investigated by García-Mina et al (2004). The usefulness and efficiency of metal-humic complexes as valuable micronutrient sources in soil has been widely debated in the literature and these experiments were designed to address the issue. Cu-, Fe- and Zn-humic substances of known stability and solubility were used as well as an unmodified sapric peat humic extract (García-Mina et al 2004). Results from this investigation suggest that the unmodified extract did not cause any significant change in Cu or Zn content in either plant species growing on three different soils. Wheat plants grown in a Fe-deficient soil (Mendigorría) demonstrated significantly greater Fe content when compared to controls when the unmodified extract was added (García-Mina et al 2004). This indicates that Cu- and Zn-humic complexes can supply the respective micronutrients in plant-available forms to deficient soils and supports the notion, at least in part, that improved uptake of micronutrients can explain the effect of humic substances on plant development.

Humic substances contribute to soil organic carbon (SOC)

Recently, much interest has been generated by “green” manures and their potential benefits to agriculture. These crops are grown in conjunction with a desired crop as a means of adding nutrients and organic matter to a soil. They are grown for a specific period before being ploughed under the soil to provide a nutrient pool. Ramesh & Chandrasekaran (2003) reported that adopting a cropping system incorporating *Sesbania rostrata* as a green manure in the sequence *S. rostrata*-rice/*S.rostrata*-rice/*S. rostrata* led to a 10.63% increase in soil organic carbon compared to traditional practice (control). Greater fulvic and, to a lesser extent, humic acid fractions in the green manure treatment were recorded (Ramesh & Chandrasekaran 2003). The traditional view has been that yield improvements from green manuring could be explained by enhanced N availability alone but this work suggests that improved yield could, at least in part, be a consequence of increased availability of soil organic carbon.

Indirect mechanisms of humates on plants

Humic substances can mediate degradation and / or inactivation of toxic substances within soils

Soil contamination by heavy metals can be reduced by phytoremediation. This method exploits the capacity of plants to extract metals in the soil by root uptake. Halim et al (2003) investigated the effect of the application of humic acid to a mineral and an organic soil on the availability of the heavy metals Cu, Pb, Cd, Zn and Ni, which are relatively phytotoxic at low concentrations in soils. Soluble and exchangeable forms of metals generally became less extractable with increasing concentrations of applied humic acid and soil ageing time (Halim et al 2003). This indicates application of exogenous humic acid can have implications for reducing heavy metal mobility within a soil and this method could be adopted as a preventative measure against heavy metal pollution of water bodies. Conversely, plant-available metals (extracted with diethylentriaminepenatacetic acid, DPTA: a mimic of plant root exudates) were generally found to increase with greater humic acid applications (Halim et al 2003). A greater proportion of metals were recovered from the organic soil because this type of soil has higher native organic matter content, which facilitates the formation of metal-humic complexes (Halim et al. 2003) suggesting that supplying soil with exogenous humic substances may keep metal in more readily available plant forms and the presence of existing soil organic matter can serve to enhance this.

Similarly, Murillo et al. (2005) investigated the effects of humic substance application on polluted soil. A fulvic-acid rich amendment was added to soils contaminated with trace metals by a mining accident in the Guadiamar river valley, SW Spain. The plant used to test the efficacy of the fulvic-acid rich amendment was the wild olive (*Olea europaea* L. var. *Sylvestris* Brot.); an evergreen tree typical of the region. ‘Alpechin’ (olive mill wastewater) was used to provide the fulvic-acid rich irrigation treatment. Mobilization of As and a fixation of Cd and Pb was observed with amendment application (Murillo et al. 2005). However, no plant uptake to phytotoxic levels was recorded. Interestingly, a general improvement in growth was observed in olive trees in these treatments, being more pronounced in plants growing on contaminated soils and the authors conclude that humic-substance application to eroded and polluted soils can be beneficial due to their ability to reduce harmful mobilization of metals (Murillo et al. 2005).

Evidence for a hormone driven mechanism and conclusion

Many claims are made as to the benefits of humates on crop yield and quality but these are often commercial claims made with little supporting evidence from unbiased laboratory experiments. There are inherent difficulties with investigating, on a wide range of economically important crops, the effects of humates, given the inherent variability of their composition and the method of application.

The stimulatory effects that have been reported on plant development are attributed historically to their nutrient content and, primarily, direct changes in root development and architecture (Vaughan and Malcolm, 1985). It is recognised that auxin, a key phytohormone, is a regulator of root development (Laskowski et al 1995). During the initiation and emergence phases of lateral root development both root basipetal and leaf acropetal auxin transport are required (Casimiro et al 2001; Bhalerao et al 2002). Recent work carried out by (Canellas et al. 2000) detected the presence of auxin groups in HA extracted from earthworm compost and this fraction has been shown to induce the proliferation of sites of lateral root emergence in maize roots (Canellas et al 2000). Although HA are regarded as macromolecules incorporated of long alkyl chains containing aromatic groups, the occurrence of hydrophobic clusters in their supramolecular structural conformation also has been described (Schulten and Schnitzer 1995; Clapp and Hayes 1999). The presence of intrinsic small bioactive molecules such as IAA clustered within the HA supramolecular arrangement might be related to both the induction of root mitotic sites and H^+-ATPase activation (Cannelas et al 2002). Previously, IAA was also detected by immunoassay in humic substances extracted from other sources (Muscolo et al, 1998). However, the separation of IAA from HA by GC means that this small molecule can be released by polarity changes in the HA microenvironment. In field conditions, such changes of polarity can occur by interactions between soluble HA and root exudates therefore observed effects of humic substances on plant performance, through changes in root development, might be hormone driven (Nardi et al 2000; Cozzolino et al 2001). It is therefore plausible that like endogenous auxins (Ruck et al 1993; Goldsmith 1993; Abel et al 1994), humic acid groups may bind to plant receptors leading to the activation of transcription factors and protein synthesis altering the activity of particular enzymes like the PM H^+-ATPase. These enzymatic changes may result ultimately in increased cell activity and tissue differentiation resulting in root growth.

The finding that humate substances contain plant hormone mimics linked responsible for changes in root initiation and development offers one possible mechanism for observed increases in crop performance through increased nutrient uptake. However, it is likely that, given the multi-component nature of these substances and the fact that those components change over time, humate related products could act in numerous direct and indirect ways on crop productivity or indeed not at all. If these substances are to be employed in UK horticulture and agriculture it will be necessary to more clearly define the components of humate products, identify their active components and establish their efficacy in economically important UK crops.

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Development of electronic nose for real-time detection of economically relevant pests and pathogens in UK crop production.

Meeting attendees at Lancaster Environment Centre, Bailrigg, Lancaster University:

Dr Tim Gibson (Scensive Technologies Ltd)

Dr Jason P. Moore (LEC)
Dr Nigel D. Paul (LEC)
Dr Jane E. Taylor (LEC)
Prof. Nick Hewitt (LEC)

Aim: The purpose of the meeting was to determine the technical and economic feasibility of novel electronic nose systems for the 'real-time' detection of crop pests. This would allow more targeted use of synthetic pesticides, or biological control, for the purpose of reducing environmental and human health problems, while substantially reducing pesticide associated input costs for growers.

Context of the project: The large-scale use of synthetic pesticides has been taking place for two and a half generations. Many of these commercially available compounds are designed to kill economic pests and non-pests alike; usually in a non-specific manner. Estimates that less than five percent of pesticide formulations by volume reach intended target organisms may well be accurate, considering the inevitability of drift and routine pesticide use as prevention without prior confirmation of infestations (WHO). The World Health Organization (WHO) estimates that 200,000 people are killed worldwide, every year, as a direct result of pesticide poisoning, up from 30,000 in 1990. The WHO further estimates that at least 3 million persons are poisoned annually, many of whom are children. Recent research has also established that trace amounts of pesticides can cross the placenta from mother to foetus with unknown long-term effects on child development[1], links between pesticide usage and altered breast development in young girls[2], increased likelihood of developing Parkinson's disease in both occasional (9%) and occupational users (43%)[3] and reduced male fertility[4]. As a direct result of increasing concerns regarding pesticide related human health issues, their associated socio-economic impacts on communities worldwide and the burgeoning cost to farmers of application (estimated to be $30bn worldwide) there is obvious global market potential in technology that can substantially reduce usage while maintaining efficacy.

All the worlds' major crop plants emit volatile organic compounds (sometimes referred to as 'call for help' volatiles; VOC's) over a wide geographical area within minutes or hours of being attacked by economic pests and pathogens. The generation of these compounds, if continually monitored, can provide early warning of crop damage and allow growers and farmers to reduce the use of pesticides through a much more targeted approach to pest management. The purpose of this scoping project is to determine the technical and economic feasibility of novel electronic nose systems for the ‘real-time’ detection of crop pests that would allow more targeted use of synthetic pesticides, or biological control, for the purpose of reducing environmental and human health problems, while substantially reducing pesticide associated input costs for growers.

The electronic nose

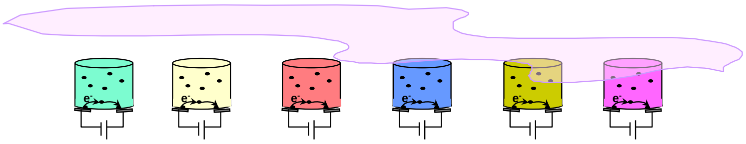
Traditionally gas chromatography and mass spectrometry have been used to analyse gas mixtures, but these techniques are too expensive and elaborate to provide a practical diagnostic test in a UK horticultural setting. Research over the past decade has resulted in the development of gas sensing devices employing discotic liquid crystal (DLCs) coatings and a unique technique for extracting data relating to individual volatiles in a mixture from both DLC and conducting polymer sensors. These Electronic nose systems are already employed in the food industry for, amongst other things, detection of ripening in coffee[5], the detection of volatiles from the MRSA bacterium in surgical environments[6] and in the diagnosis of early stage lung cancer through analysis of human exhalations[7].

Just like the animal or human nose, the electronic version uses an array of sensors that are not designed to detect any one chemical (see figure 1). Instead they respond to the overall profile of compounds in a sample (figure 1). E-noses are not quantitative devices; their power lies in their ability to ‘learn’ to recognize almost any compound, or combination of compounds, at a sensitivity of approximately < 1 part per billion. It is at this range of sensitivity that it becomes possible to extract useful plant stress generated volatile information from the environment.

a)

BASELINE RESISTANCE

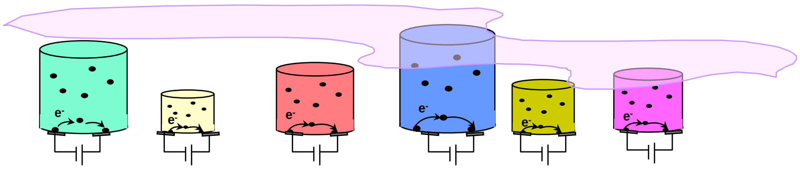
All of the polymer films on a set of electrodes (sensors) start out at a measured resistance, their *baseline resistance*. If there has been no change in the composition of the air, the films stay at the baseline resistance and the percent change is zero



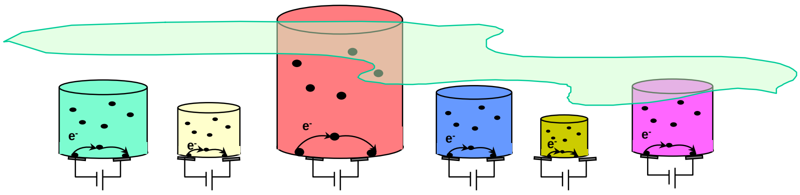
b)

THE ELECTRONIC NOSE SMELLS SOMETHING

Each polymer changes its size, and therefore its resistance, by a different amount, making a pattern of the change



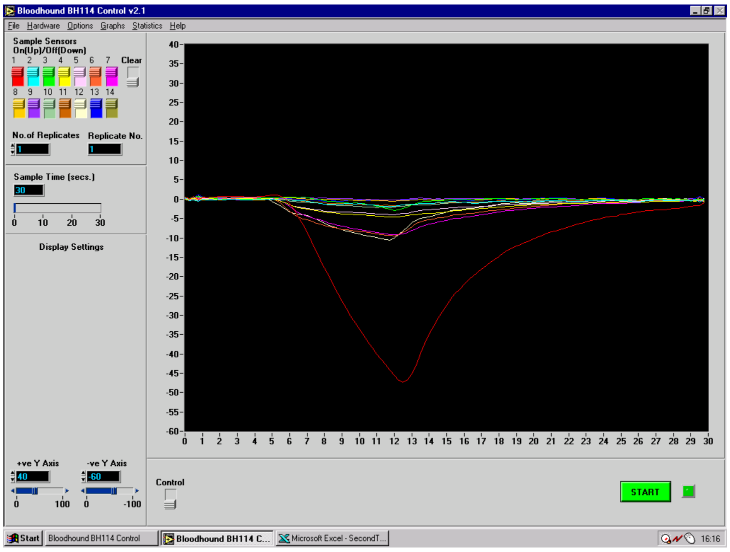
If a different compound had caused the air to change, the pattern of the polymer films' change would have been different:



The E-nose uses a collection of different polymer films. These films are specially designed to conduct electricity. They start at a baseline resistance (a) but when a substance -- such as a stray volatile molecule is absorbed into these films, the films expand slightly, and that changes how much electricity they conduct (b). Because each film is made of a different polymer, each one reacts to each substance, or analyte, in a slightly different way (b). And, while the changes in conductivity in a single polymer film wouldn't be enough to identify an analyte, the varied changes in a number of films produce a distinctive, identifiable pattern.

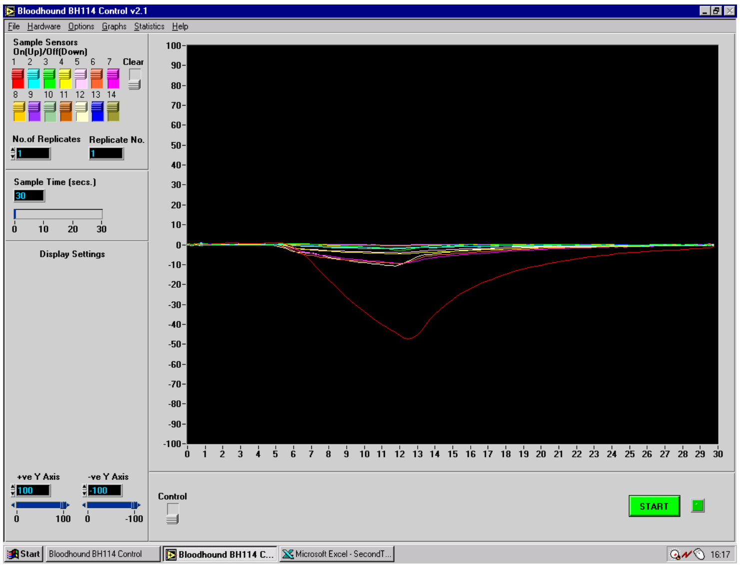
Demonstration of E-nose at Lancaster Environment Centre

A small-scale demonstration experiment was carried out during Tim Gibson's visit to LEC using their generic BH114 E-nose. A volatile profile for a 6 week old tomato plant challenged with Spider-mite (fig. 2.a) and a non treated control (fig. 2.b) were characterised from the base and top of the developing canopy. In a second experiment the VOC profile of a tomato plant inoculated with mildew (fig. 2.c) and a non-treated control (fig. 2.d) were also characterised. Following Dynamical factor analysis (DFA) of the raw data clear differences in the volatiles profile cluster of each control and respective Spidermite and Mildew treatments were observed (figure 3).



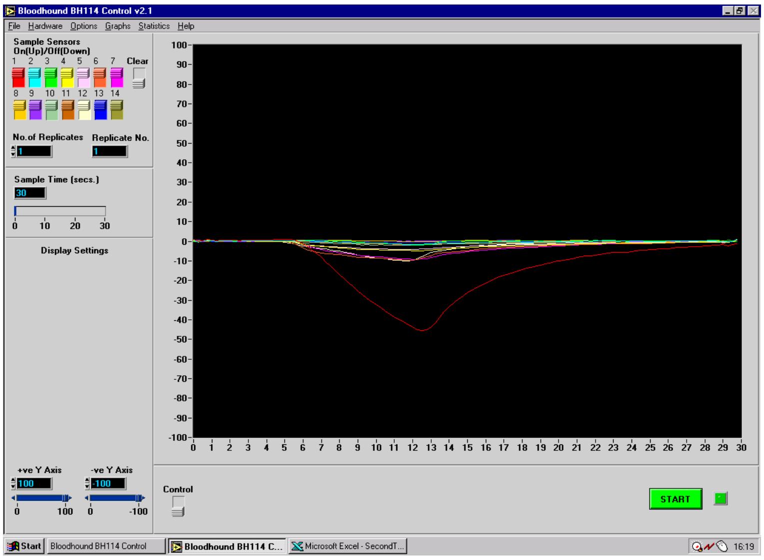
No.of Replicates 1
Replicate No. 1
Sample Time (secs.) 30
+ve Y Axis 40
-ve Y Axis 60

Figure 2.a. Volatiles profile from treated (Spider-mite challenged) in 6 week old tomato plants.



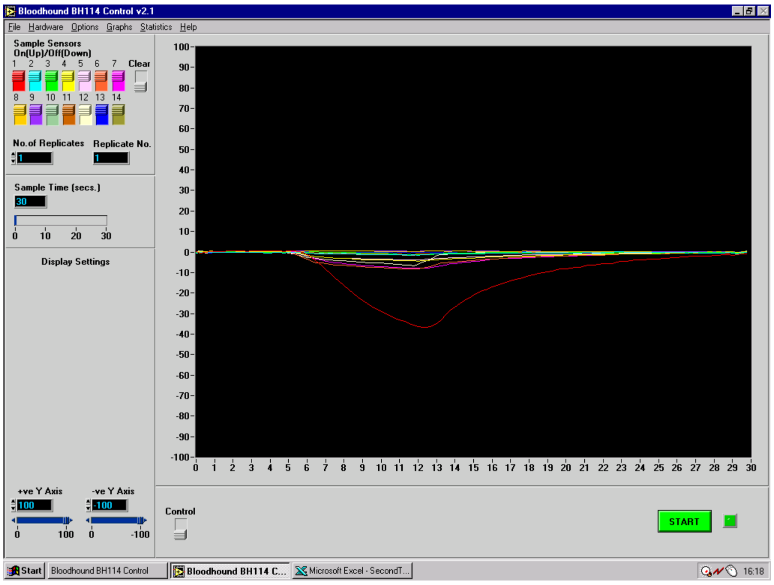
No.of Replicates 1
Replicate No. 1
Sample Time (secs.) 30
+ve Y Axis 100
-ve Y Axis -100

Figure 2.b. Volatiles profile from Control (no spider mites) in 6 week old tomato plants.



No.of Replicates 1
Replicate No. 1
Sample Time (secs.) 30
+ve Y Axis 100
-ve Y Axis -100

Figure 2.c. Volatiles profile from treated (Mildew inoculated) 6 week old tomato plant



No.of Replicates 1
Replicate No. 1
Sample Time (secs.) 30
+ve Y Axis 100
-ve Y Axis -100

. Figure 2.d. Volatiles profile from Control (no mildew) 6 week old tomato plant

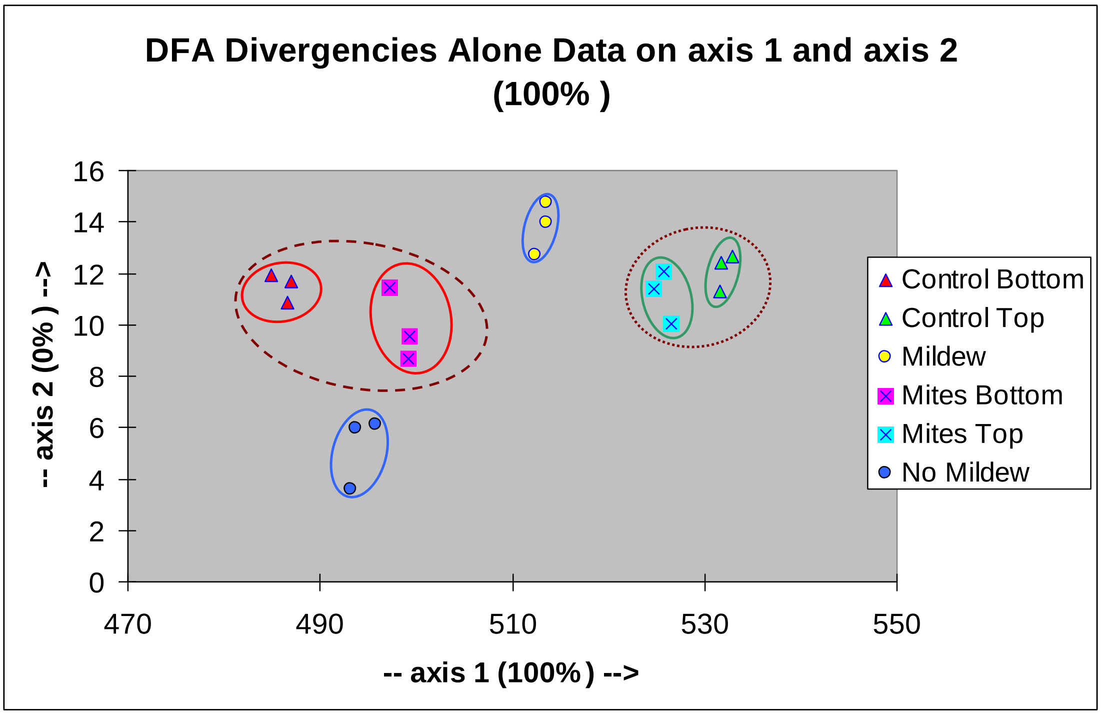


Figure 5. Shows clustering of VOC profiles taken from Spider mite control at the base of the plant (▲) Spider mite treatment at the base of the plant (☒) Spider mite control at the top of the plant (△) Spider mite treatment at the top of the plant (☒) Mildew infected plants (⬣) and Mildew control plants (⬣).

Possible routes forward

This demonstration experiment used a generic 'off-the-shelf' e-nose system to successfully characterise the difference in 'smell' of plants that had been challenged with a pest and pathogen common to UK tomato crops.

In order to successfully transfer this technology from the lab to, in the first instance at least, a commercial glasshouse setting a necessary first step would be to identify the specific emitted volatiles of interest using traditional GCMS technology. Once identified it would then be possible to develop (a) pest specific volatile reacting polymers to more easily discriminate those volatiles in a 'noisy' commercial glasshouse and (b) a sampling technology that could be readily used by crop managers.

Funding

- The GCMS work would be best carried out in a six month scoping project at LEC costing £10-£15k.
- The hardware development and commercial exploitation phase would require LINK funding in the region of £300k (estimate).

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Papers published

A demonstration of the potential benefits of modification of light spectral quality in horticultural crops

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Abstract

There are now a range of horticultural cladding plastics that alter the spectral balance of sunlight reaching the crop. We studied the responses of a range of horticultural crops to such plastics. A plastic which increased the ratio of red: far red reaching the crop provided effective growth regulation in many crops, but reduced growth is clearly undesirable in crops where biomass yield is the key agronomic output.

Keywords: ultraviolet radiation, spectral modification, red:far red ratio, plant biomass, crop quality, herb oils.

INTRODUCTION

The range of crops cultivated under relatively-low cost plastic covered structures is rapidly increasing in UK horticulture because protection delivers an extended growing season, improved crop scheduling and increased quality of produce. Cultivation under covers in itself delivers a range of quality benefits in terms of prevention of soil splash and reduction in leaf wetness, both of which can in turn lead to reduced disease. However, advances in plastics technology have allowed the manufacture of novel materials that ‘fine-tune’ the growing environment still further, by manipulating the quantity and wavelength of light reaching the crop. Much of the international research on modified plastics has focussed on either (a) the manipulation of the red : far red ratio to provide plant growth regulation (e.g. Clifford et al., 2004; Runkle et al., 2002) or (b) modification of the UV spectrum for pest or disease control (reviewed by Raviv and Antignus, 2004). The latter approach has mostly used cladding plastics with reduced UV transmission to inhibit sporulation of pathogens or interfere with the foraging behaviour of insect pests. However, there is a fundamental literature on plant UV responses which suggests that increasing penetration of UV through horticultural plastics might deliver benefits in terms of growth regulation, induction of host resistances against pests and pathogens, and alterations in crop chemistry such as pigmentation or essential oil concentration (Paul and Gwynn-Jones, 2003). We describe here a project that aims investigate the costs and benefits to the UK horticultural industry of adopting modern plastic technology. The project evaluates cladding plastic with contrasting light manipulating properties for use in the production of a range of UK horticultural crops and seeks to rapidly transfer this knowledge to UK growers (Anon, 2005).

MATERIALS AND METHODS

The experimental facility is at Stockbridge Technology Centre UK, and consists of five commercial structures, each covering an area of 740m^2, and a comparable, uncovered field plot. We report here responses to four different plastics:-

1. Standard clear horticultural film
2. UV-B transparent film (designed to transmit the full solar UV spectrum)

3. UV opaque film
4. Red / far red modified film (designed to increase R:FR ratio)

All films are 150 µm thick polyethylene, with specific spectral transmission properties produced by specific additives. The standard clear horticultural film (Fig 1) is a commercial horticultural film with >90% transmission across the PAR range (400-700nm) but rapidly decreasing transmission in the UV (280-400nm), with less than 10% transmission below 350nm. The UV-opaque film has comparable PAR transmission to the standard film, but has a total UV-A (320-400nm) transmission of only 10% and its UV-B (280-320nm) transmission is zero (Fig. 1). Transmission through the UV-transparent film is >90% PAR, and > 80% across the whole of the solar UV range from 290 – 400 nm (Fig. 1). The red:far red modified film radiation has a red:far red transmission ratio of approximately 8.8 due to its low transmission of FR (approx. 4% between 720 and 740nm). This film also has reduced transmission of longer PAR wavelengths, so its total PAR transmission is lower than in the other three films (c. 75%: see Fig 1). The spectral properties of the films have proved broadly stable over two growing seasons (Paul et al., 2005).

The overall project examines the effects of plastics on a wide range of crops (Anon, 2005), but we focus here on just two sectors, leafy salads and herbs, to illustrate the major effects of plastics that are applicable across many species.

Crop production

All crops were grown in the field under the plastics described above. Herbs (black peppermint, lavender, rosemary and sage) planted out from 9cm pots in spring 2003 are harvested for essential oil extraction in September 2003 and again in September 2004. Lettuce cultivar ‘Constance’ was sown in situ in three repeated experiments during 2003 and 2004, with harvests in August 2003, June 2004 and September 2004. Plants were grown using standard commercial practice and harvested at commercial maturity.

Statistical analyses

Comparisons between treatments were made using one-way ANOVA followed by post-hoc tests using Tukey’s HSD test. . All analyses were performed using SPSS v11.5 (SPSS Inc.).

RESULTS AND DISCUSSION

For herbs grown for oil extraction, harvestable yield is a function of (i) crop biomass and (ii) oil concentration per unit biomass. Previous work has suggested that oil concentration is increased by exposure to supplementary UV-B lighting in some herbs (Johnson *et al*., 1999; Karousou *et al*., 1998; Maffei and Scannerini, 2000), but we were unaware of any previous studies that had considered the balance between such effects and the reduction in biomass expected from UV-B exposure (e.g. Paul and Gwynn-Jones, 2003; Krizek et al., 1998). In our studies we found no evidence that manipulating solar UV using cladding plastics had any consistent or significant effect on oil concentration in any of the herbs studied (data not presented). However, the lack of effects on oil concentration did not mean that there was no effect of plastics on oil yield. Overall, the data obtained during the two years of these herb trials has shown the clear and substantial benefits of growing these herbs under protection, but these are driven by the effects of plastics on crop biomass. Protection *per se* gave significant increases in oil yield, even under the good growing conditions of 2003. In the cool, wet conditions of 2004 the effect was even more marked. Averaged across all crops, all plastics and both seasons protection increased total oil yield per plant (i.e. the product of both oil concentration and harvestable biomass) by 6.9 ± 1.0 fold compared with the field. Especially in the wet, dull growing conditions of 2004, protection with almost all plastics led to significant increases in yield since crops were not exposed to cold and wet growing conditions. The specific mechanism of this response appeared to vary between crops, for example, in peppermint all plastics led to almost complete avoidance of rust (*Puccinia menthae*) which defoliated the field crop. In terms of specific spectral modifications, the strong growth limiting effect of the R:FR increasing film led to consistently low oil yields, making this plastic a poor choice for growing herbs for oil (Table 1). The UV-transparent film gave good oil yields in 2004 but in 2003 it led to marked growth restrictions which limited oil yield. Given the higher light intensity in 2003 this variation in response would be consistent with a dose-dependent growth inhibition by the shorter wavelengths of solar UV, which is commonplace in many plants (e.g. Paul *et al*., 2005), but this remains to be proven. However, in the absence of any consistent increase in oil concentration under UV-T, this film does not seem a good choice of general use in herb crops grown for oil extraction. In terms of oil yield the outstanding plastic over the range of herbs and both seasons was UV-O, which delivered increases in crop biomass without loss of oil quality or concentration (Table 1). There was no evidence from any crop or season that UV-O would be inferior to standard plastic i.e. there is added benefit to be gained from using suitable spectral modification beyond that gained from simply covering the crop. This was a rather surprising conclusion given previous reports that exposure to UV might increase oil concentrations, but illustrates the risks in extrapolating from UV responses obtained using artificial lamps to spectral modification of field sunlight.

The increase in crop biomass under the UV-opaque film has been observed not only in herb species but also across a wide range of crops, ranging from asparagus to cut flower crops (Anon, 2005). Whether or not this is an agronomically desirable response depends of the requirements of specific crops. In propagation crops, greater biomass is undesirable since it is associated with less sturdy plants that appear to be more vulnerable to physical damage during transplanting. For such crops, UV-transparent or R:FR increasing films appear the best choice of plastics (Paul et al., 2005; Moore et al., 2005; Wargent et al., 2005). Where crop bulk is the major requirement, the UV-opaque film is a good choice, but other aspects of crop quality need to be taken in to account. This is exemplified by leafy salad crops. In the lettuce cultivars we have studied, production under UV-opaque film has given increases in harvestable yield averaging around 20% over the standard (Figure 2a). However, in red-leaf salads such as lollo rosso lettuce, this yield increase is at the expense of leaf colour. The leaves of lollo rosso lettuce cultivar ‘Constance’ produced under UV-O film are visually almost entirely green, with little or none of the anthocyanin pigments that produce the commercially required red coloration. Standard film has some limited red pigmentation but far less that in crops grown in the field. Use of the UV-transparent film provides the benefits of protection, which is important in the UK to prevent rain damage of this crop, without visually noticeable loss of pigmentation compared with the field. However, cultivation under UV-T consistently leads to significantly slower growth, with harvestable head fresh weight up to 40% lower than under standard film (Figure 2a). Thus, there is a trade-off between yield (biomass) and quality, certainly in pigmentation. This may not pose major problems commercially, where planting density and/or crop scheduling might be adjusted for cultivation under UV-T, for example where lollo rosso is cultivated for “baby-leaf” production rather than mature heads. However, another approach might be the “tactical” deployment of plastics to deliver specific end points at specific stages of the crop. In the case of lettuce cultivar ‘Constance’ we have grown the crop initially under UV-O but then switched plastics to UV-T towards maturity. When switched from UV-O to UV-T, reduction in harvestable biomass in this cultivar became significant only after periods of greater than two weeks (Figure 2b). By contrast, induction of anthocyanin pigmentation was rapid, with plants moved from UV-O to UV-T for only 5-7 days being visually indistinguishable from those grown continuously under UV-T. Thus, using this approach it is possible to grow for maximum yield but then combine this with maximum quality by using a different plastic just prior to harvest.

CONCLUSIONS

Manipulation of the spectral quality of light reaching the crop using plastics with specific light transmission properties brings about changes in crop growth, morphology and chemistry. Other benefits include pest and disease control, especially for UV-modifying plastics (Raviv and Antignus, 2004). No single plastic can deliver optimum yield and quality (and optimum control of all pests and pathogens) in all crops and, as we have shown with lettuce, there may be trade-offs between yield and quality that need to be considered in commercial application of spectral modification. In practice, growers will need to make informed decisions on the best choice of plastic to meet the needs of specific crops. Such informed decisions require solid understanding of the effects of different plastics based not only on comprehensive field assessments but also, we believe, effective integration between such trials and the fundamental photobiology that underpins understanding of the responses that are observed (Paul et al., 2005).

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Tables

Table 1. Oil yield per plant in four species of herb frown under different plastics. Yields are expressed relative to that of field-grown plants. Data for 2004 are replicates of three replicates ± 1SEM and, within species, plastics that do not share the same letter are significantly different at $p<0.05$. Mean increases for the 2003 growing season are included for comparison (lavender oil yield was not measured in 2003).

	Black peppermint		Rosemary		Sage		Lavender	
	2004	‘03	2004	‘03	2004	‘03	2004	‘03
Standard	5.1 ± 0.7^{d}	1.2	12.1 ± 0.7^{c}	6.0	3.6 ± 0.1^{b}	3.2	13.9 ± 0.6^{c}	-
UV-transparent	2.5 ± 0.3^{b}	1.1	21.2 ± 0.4^{d}	3.3	5.4 ± 0.4^{c}	3.2	26.8 ± 4.1^{d}	-
UV-opaque	5.6 ± 0.7^{d}	1.2	18.8 ± 1.5^{d}	5.1	$5.8 + 0.5^{c}$	6.4	24.7 ± 5.9^{d}	-
R:FR modifying	3.4 ± 0.1^{c}	0.8	6.3 ± 0.4^{b}	2.5	3.4 ± 0.1^{b}	3.2	8.6 ± 1.5^{b}	-
Field	1.0 ± 0.1^{a}	1.0	1.0 ± 0.0^{a}	1.0	1.0 ± 0.1^{a}	1.0	1.0 ± 0.4^{a}	

Figures

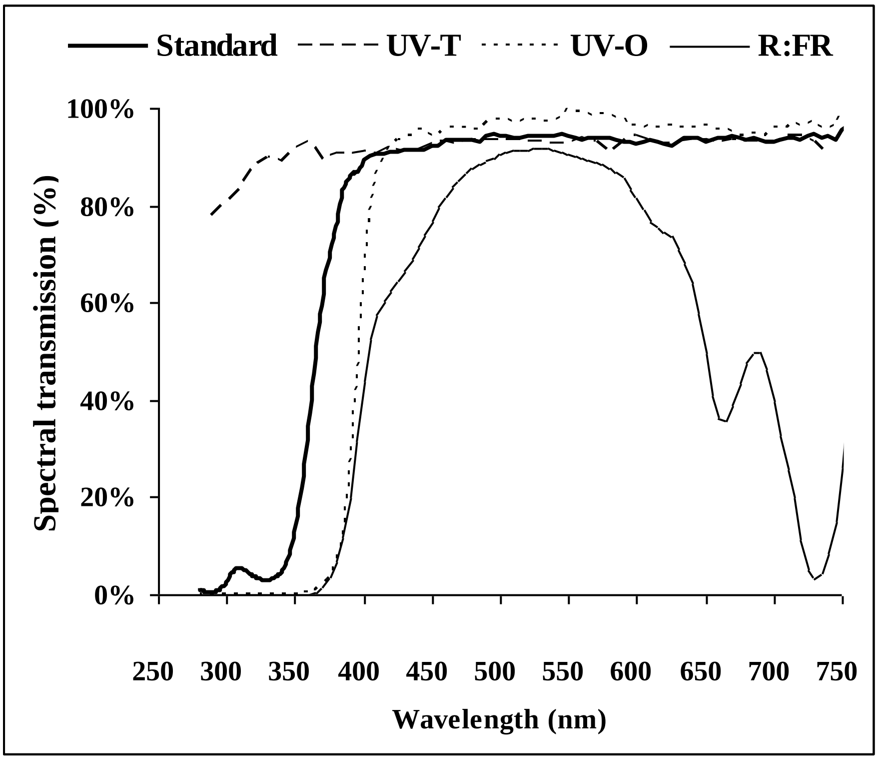

Fig. 1. Spectral transmissions of the four plastics used in these experiments. All data for new films and were determined using a double scanning spectroradiometer (Macam Photometrics, Livingston, UK) with a 75W Xenon arc lamp as a light source.

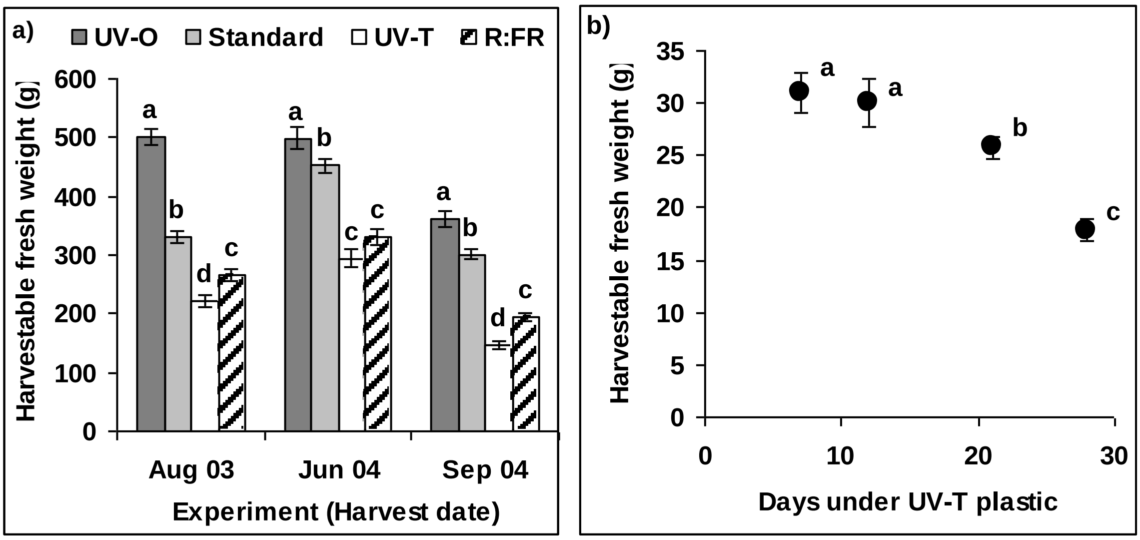

Fig. 2. a) Harvestable head fresh weight in lettuce cv lollo rosso grown permanently under different plastic. Data are means ± 1SEM of 20-25 replicates, and within harvest dates plastics that do not share the same letter are significantly different at $p<0.05$.

(b) Harvestable head fresh weight in lettuce cv lollo rosso grown initially under UV-opaque plastic and then switched to UV-transparent. Data are means of 10 replicate plants ± 1SE and treatments that do not share the same letter are significantly different at $p<0.05$.

Manipulation of light spectrum for crop growth regulation

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Keyboards: Spectral modification, propagation crops, field trials.

ABSTRACT

Crop plant growth responses to light spectral quality can be exploited to deliver a range of agronomically desirable end-points. This can be achieved using a new generation of plastics with specific spectral properties as crop covers. Over a period of two UK growing seasons we have investigated the potential of the following four plastics a) a widely used commercial Standard clear b) a filter that is largely UV transparent c) a UV blocking filter and d) a filter that increases the ratio of red: far-red radiation in manipulating plant growth for commercially desirable end-points in propagation lettuce and two brassica crops. At the end of the propagation stage both plant height and total leaf area was significantly reduced under the UV-transparent and red: far red films in lettuce and cabbage but not cauliflower. Final yields of cauliflower and lettuce from plants propagated under the UV-transparent film were significantly higher than from standard. The R:FR increasing film gave higher yields than standard in lettuce, but in cabbage yields were significantly lower in plants from the R:FR film. The mechanisms of these responses are discussed.

INTRODUCTION

The cultivation of crops under simple plastic covered structures is now commonplace in UK horticulture because of its potential to extend growing seasons, control harvests and improve the quality of produce. In recent years advances in technology have allowed the manufacture of novel materials that ‘fine-tune’ the growing environment still further, by manipulating the intensity and wavelength of light reaching the crop. The use of spectral filters to increase the ratio of red : far red (R: FR) reaching the crop has been shown to deliver effective growth regulation in a number of crops (e.g. Clifford et al., 2004; Cerny et al., 2003; Runkle & Heins, 2002). The growth regulatory effects of the UV-component of sunlight are well defined (Krizek et al., 1998; Paul & Gwynn-Jones, 2003) but their commercial exploitation has rarely been explored (Hoffman, 1999). Plastics that block all solar UV radiation from reaching the crop are used commercially as an element of pest and disease control (Raviv & Antignus, 2004), but their effects on crop growth or morphology appear to be poorly defined.

We are currently investigating the use of a range of spectral filters to deliver commercially desirable end-points for a range of UK crops (Anon, 2005; Paul et al., 2005). Here we report the effects of spectral modification on the propagation phase of cabbage, cauliflower and iceberg lettuce. The commercial aim of growth regulation during propagation of these crops is to produce compact plants that withstand mechanical handling during transplanting to the field and go on to give good yields. Thus, we assess post-transplantation performance of plants produced under different plastics.

MATERIALS AND METHODS.

The facility

Experiments were carried out at Stockbridge Technology Centre (STC: 53N 1W) using a series of commercial “high-tunnel” structures (Haygrove Tunnels Ltd, Ledbury UK), as described before (Moore et al., 2005). Each spectral filter structure covers 740m^2 over 4 individual bays, each measuring 3m high x 6m wide x 30m long. We report here responses to four different plastics:-

5. Standard clear horticultural film
6. UV-B transparent film (designed to transmit the full solar UV spectrum)
7. UV opaque film
8. Red / far red modified film (designed to increase R:FR ratio)

See Moore et al (2005) for more details.

Plant material

Lettuce

Plants of Iceberg lettuce (*Lactuca sativa* L. cv. Challenge, Syngenta seeds Ltd) were raised for 14d from sowing using a widely employed UK commercial practice at Crystal Heart Salads (Holme-on-spalding-Moor, UK). Briefly, seeds were germinated in 4cm^3 peat blocks (Fison B2 Blocking Compost, Fisons, UK) at 16 $\underline{+}$ 3°C in the dark for 4d before being transferred to commercial glass for a further 10d. At 14d plants were transferred to STC and randomly distributed under the four filter treatments for a period of 14d at which completed the propagation stage.

C*abbage and Cauliflower*

Propagation cabbage (*Brassica oleracea* cv. Summer green) and cauliflower (*Brassica oleracea* cv. Thasca) were raised using widely recognised commercial practices. Briefly, seeds were germinated in 3cm^3 blocks (Fison B2 Blocking Compost, Fisons, UK) under commercial glass at 30 $\underline{+}$ 5°C / 15 $\underline{+}$ 5°C day/night temperature under natural daylight (no supplementary lighting) for 14d before being transferred to filter treatments for 28d prior to planting out.

Determination of final leaf areas

Leaf area at the end of the propagation phase was measured following destructive harvests using an automatic Leaf Area Meter LI-3000 (Li-Cor, Inc., Lincoln, NE, USA).

Field trials

All field trials took place using a random block design at STC. Following harvesting fresh weights were determined within 1 hour.

Statistical analysis

Multiple Student t-tests were used in all analysis. All analyses were performed using Sigmastat V 2.03 (SPSS Inc.).

RESULTS

Leaf area and plant height at the end of the propagation phase

In lettuce and cabbage leaf area at the end of the propagation period in plants grown under UV transparent or R: FR were significantly lower than in plants from the standard film (P<0.001), but were not significantly different from each other (Fig. 1). Cultivation under the UV-opaque film has no significant effect on leaf area in this experiment, but did cause significant increases in leaf area in other, comparable experiments (Paul et al., 2005). There was no significant effect of filter treatments on total leaf areas in cauliflower (P>0.05, Fig. 1).

At the end of the propagation stage of both lettuce and cabbage plant heights were significantly lower under UV transparent or R: FR than standard film (p<0.01) and, unlike leaf area, the effect of increasing R:FR was significantly greater than that of the UV-transparent film (Figure 1). In lettuce plant height was significantly greater under the UV-opaque film than any other treatment (Figure 1). In cabbage, the UV-opaque film had no effect on plant height. In cauliflower, the only significant difference from the standard film was an increase in plant height under the UV-opaque film (Figure 1).

Yield at harvest

In lettuce, all three spectrally modified plastics gave significantly greater yields than plants propagated under the standard film (Figure 3). Increases ranged from 18% (UV-opaque) to 33% (R:FR increasing film). By contrast, in cabbage plant propagated under UV-opaque or the R:FR increasing film produced significantly lower final yield than plants propagated under standard plastic (by 27 and 21% respectively: Figure 3). Propagation under the UV-transparent film had no significant effect on final yield. In Cauliflower, propagation under the UV-transparent film slightly (8%) but significantly increased harvestable fresh weights compared with the standard film (Figure 3). Final yields from plants propagated under the R:FR increasing or UV-O films were not significantly different from the standard film.

DISCUSSION

Our data show that spectral modification for even limited periods (14 days for lettuce and 28 days for the brassica crops) can significantly alter plant growth and morphology. The growth regulatory effects of plastics that increase R:FR ratio have been demonstrated in a range of crops, although mostly ornamentals (e.g. Clifford et al., 2004; Cerny et al., 2003; Runkle & Heins, 2002). The significant reductions in leaf area and stem elongation observed in lettuce and cabbage are consistent with these previous reports, but it was notable that cauliflower did not show the expected responses. The lack of response to increasing R:FR is not unique in our experiments, where the R:FR modifying film has been found to have no significant effect, or even to increase plant height in some subjects, not only at the propagation stage (e.g. in some bedding plants) but also in crops grown to maturity under the plastics (e.g. lilies grown for cutflowers, and asparagus). Such responses to a film that increases in the ratio of R:FR light very substantially are surprising, but may be a function of the overall spectral properties of the film. The R:FR increasing film used in our studies is also UV-opaque (Moore et al, 2005; Paul et al., 2005) and the responses we observed my be a function of conflicting effects of increasing R:FR and reducing UV radiation. Cultivation under UV-opaque film tends to increase leaf area and plant biomass compared with standard film, which may be desirable in some crops (Moore et al., 2005). For growth regulation, cultivation under UV-transparent film delivers reduced stem elongation and leaf area, as here is lettuce and cabbage, across a range of species. Thus, our data show that modification of the UV component of the solar spectrum can have major effects on crop growth and morphology. Unlike R:FR modification, the use of UV-transparent film for growth regulation in protected crops is little studied (but see Hoffman, 1999). However, the basic photobiological literature suggests that exposure to solar UV will tend to reduce leaf expansion and stem elongation, and increase branching, in a wide range of species (e.g. Paul & Gwynn-Jones, 2003). Data from our wider studies show that such responses are no less consistent than those to R:FR modification.

In the case of the propagation crops studied here growth regulation is required commercially to minimise plant damage during handling and transplanting in to the field, and to maximise growth and final yield. Both R:FR increasing and UV-transparent films delivered the growth regulation required. The plant characteristics produced under these plastics met the primary requirement of UK propagators for more compact, “stockier” plants, and commercial growers are already adopting structures clad with UV-transparent film as delivering superior plants at a lower cost than conventional glasshouses. Our field assessments suggest that propagation under UV-transparent film can lead to significantly greater final yield of lettuce and cauliflower than production under standard plastic, and at least no detrimental effect in cabbage (Figure 3). By contrast, the growth-regulating effects of propagation under the R:FR increasing film was not translated in to increased final yield. In fact brassica plants propagated under the R:FR film gave significantly lower yields than those produced under UV-transparent film (Figure 3). This highlights the broader point that post-transplant performance can not be predicted simply from the gross morphology of plants at the end of the propagation stage. Propagation under the UV-transparent film appears to cause changes contributing to long-term performance that are not produced by increasing R:FR ratio. Our initial data show that increasing leaf thickness is one such specific response to UV-exposure (Wargent et al., 2005), but other known effects of UV on physiology or biochemistry may be equally important to final yield. Such mechanisms, and multi-season assessments of field performance are the focus of continuing research.

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FIGURES

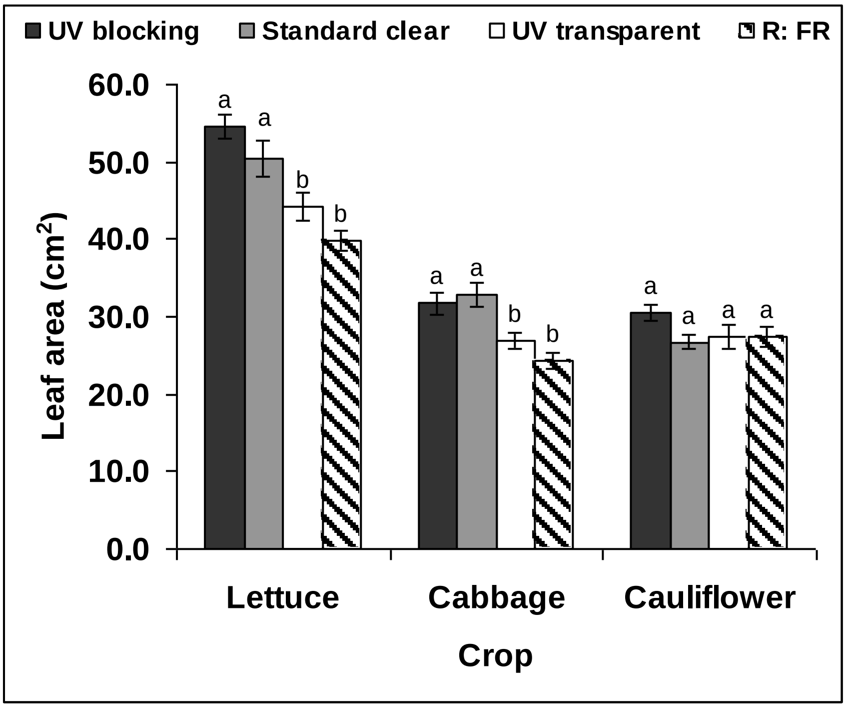

Figure 1. Effect of treatments on total leaf area at the end of the propagation stage in lettuce, cabbage and cauliflower. Each value is the mean ± SE of 20 replicates.

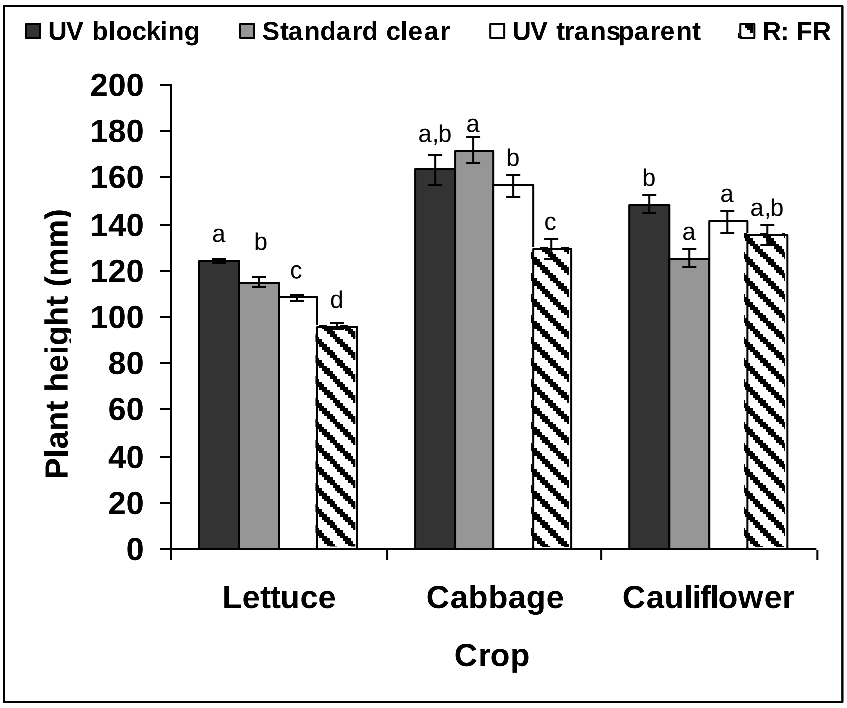

Figure 2 Effects of treatments plant height at the end of the propagation stage in lettuce, cabbage and cauliflower. Each value is the mean ± SE of 20 replicates.

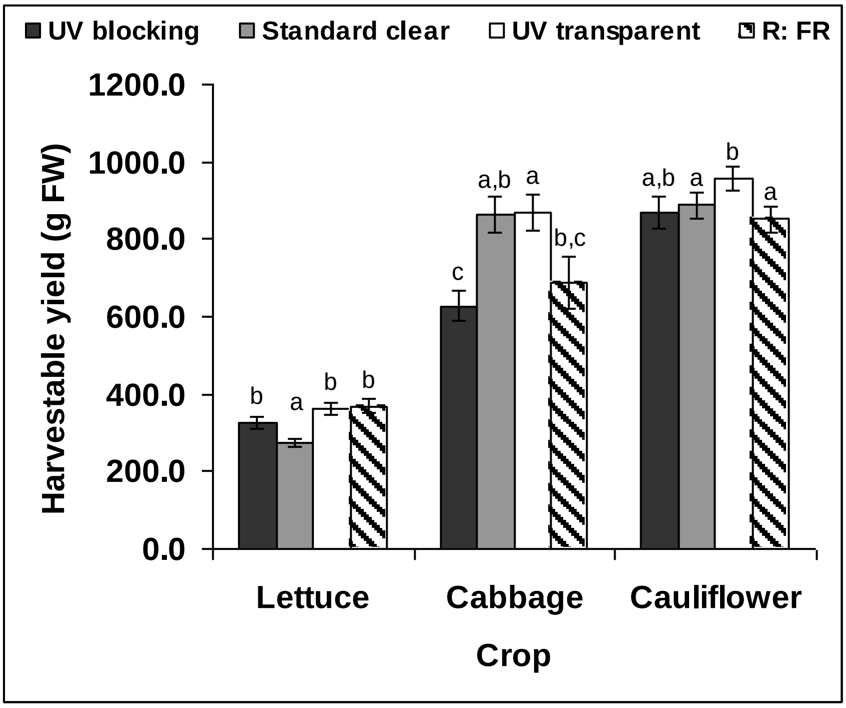

Figure 3. Effect of treatments on harvestable fresh weights (g) in lettuce, cabbage and cauliflower in the 2004 growing season. Each value is the mean ± SE of 20 replicates for cabbage and cauliflower and ≥ 26 for lettuce.