Examining the impact of oxygenized[tm] water

(1) on the establishment, growth and yield of horticultural/ agricultural crops and

(2) its potential use for the decontamination of freshly-harvested produce

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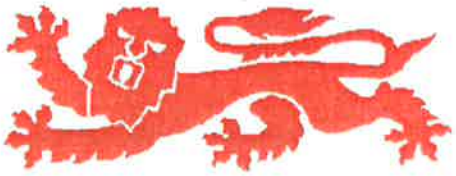
A **confidential report** for the period early February- end March 2002

University of Newcastle upon Tyne



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This report to Water Technology, details a series of experiment conducted at the Air Pollution Laboratory within the Department of Agricultural and Environmental Science at Newcastle University between early February and end March 2002 i.e. the first 2-months of a 6-month 'seedcorn' project.

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Part 1: Establishment, growth and yield of horticultural/agricultural crops

Pavlina Drogoudi and Jeremy Barnes

1.1 Background Information

1.1.1 Oxygen is required for seed germination

Successful crop establishment requires seed of high quality and vigorous germination. Seeds of many horticultural/ agricultural crops exhibit poor and variable germination. This is at least in-part due the poor oxygen-uptake characteristics exhibited by the seed coat. Incubation of seeds in an oxygen-rich atmosphere has been shown to stimulate germination in some species e.g. lettuce, sunflower, radish, carrot, *Sinapis arvense* (weed), *Eriochloa villosa* (weed),.*Rumex crispus* (weed), *Bidens pilosa* (weed) *and Tilia americana* (basswood tree) (Mayer and Poljakoff-Mayber, 1977). The seed coat either limits the supply of oxygen to the embryo and prevents germination directly or prevents the oxygen-mediated removal of germination inhibitor(s) from the embryo. It is known that high oxygen tension reduces the amount of extractable inhibitor, presumably as result of oxidative degradation. For example, the seed coat ~~of *Sinapis arvense*~~ is permeable to water but less to oxygen, so removal of the seed coat allows an adequate supply of oxygen to reach the embryo; slowing down inhibitor formation and promoting germination (Table 1).

Table 1. Effects of O_2 concentration on O_2 uptake and growth inhibitor production by *Sinapis* embryos (Mayer and Poljakoff-Mayber, 1977).

O_2 concentration (atmos.)	*O_2 uptake (ml g^{-1} 4 h)*	*Inhibitor content*
0	0	11.8
0.05	1	10.5
0.1	2	9.2
0.2	2.5	8.8
1	2.5	8.5

1.1.2 Oxygen promotion of root growth

Oxygen limitations in the rhizopshere are a common cause of restricted plant growth/development. Indeed a close relationship exists between the rate of oxygen consumption by roots (ie root respiration), plant relative growth rate and anion uptake (Figure 1.1) (Lambers *et al.,* 1998). Root respiration commonly accounts for 10-50% of the total carbon assimilated each day *via* photosynthesis and represents a major cost in terms of the plant's daily carbon budget. The relative cost is much greater in slow-growing plants than fast-growing plants. Raising the oxygen concentration in the rhizosphere through the addition of oxygenized™ water might be expected to stimulate ion uptake through effects on (i) the form/mobility of nutrients in the soil, (ii) the rate of root respiration and (iii) specific respiration costs associated with ion uptake (i.e. increasing the efficiency of ion uptake). Effects associated with the improvement of plant performance in terms of growth rate/yield.

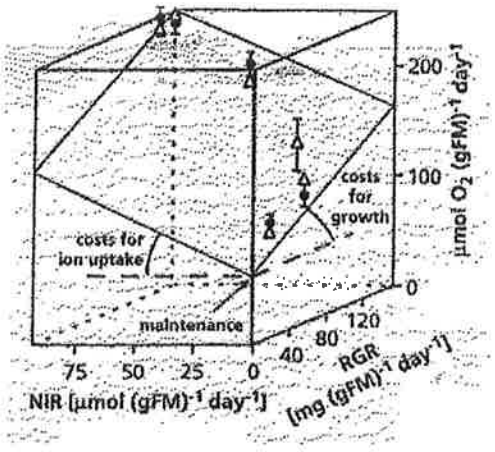

Figure 1.1 Relationships between the rate of O_2 consumption (µmol g^{-1} FM day^{-1}) by roots, their relative growth rate (RGR) and their net rate of anion uptake (NIR). The plane gives the predicted mean O_2 consumption. The intercept of the plane with the y-axis yields the rate of O_2 consumption by roots required for maintenance (Lambers *et al.,* 1998).

1.2 Aims and Objectives

Measurements were designed to investigate the following within the reporting period February- March 2002

- the application of oxygenized™ water to improve the rate and uniformity of germination in vegetable, salad and flower species, and
- the application of oxygenized™ water to improve the growth, development and yield in crops grown in soils of contrasting nutrient status.

1. 3 Materials and Methods

1.3.1 Oxygenized™ water

Oxygenized™ water was produced using a system incorporating a water softener (2-day recharge cycle), a 40 gallon mixing/stabilization tank and a chlorine scubber (GK55 filter) – all fitted and maintained by Phil Leslie (incl. fornightly inspection/checks).

Measurements of temperature, conductivity, oxygen redox potential and dissolved oxygen were made using an Aquanode ES controller (Aquadyne Computer Corp., California, USA). Total hardness, total chlorine, free chlorine and total alkalinity were measured with an AquaCheck test strip (Environmental test systems, Inc. Elkhart, USA). Full spectrum analysis of water samples pre- and post-oxygenization are awaited following the despatch of samples to anlaytical laboratory advised by Phil Leslie.

1.3.2 Seed germination

Seed germination was investigated for (a) vegetable and salad crops: onion (*Allium cepa* C. var. Bedfordshire Champion and var. Senshyu yellow), aubergine (*Solanum melongena* L. var. Honeymaker), cabbage (*Brassica oleracea* L. var. Christmas drumhead), okra (*Hibiscus esculentus* var. Clemsons spineless), lettuce (*Lactuca sativa* E. var. Lollo Rosa), parsley (*Petroselinum crispum* var. Champion Moss curled 3), carrot (*Daucus carota* var. St. Valery), and (b) ornamental crops such as *Ageratum houstonianum* var. Blue Mink and *Rudbeckia hirta* L var. Marmalade.

Seeds were placed on two layers of Whatman filter paper No 1 placed in a 9-cm diameter petri dish. Each Petri dish was irrigated with 6 ml of tap or oxygenized™

water daily, having first removed the existing water with the aid of a pipette. Petri dishes were covered with aluminium foil and incubated in controlled environment chambers providing a maximum daily temperature of 25 ^{0}C.

Germination was recorded daily and the percentage germination was calculated. A seed was recorded as 'germinated' on the appearance of a radicle. Germination vigour was calculated as described by Bradbeer (1988):

$$\text{Vigour} = \left(\frac{a/1 + b/2 + c/3 + d/4}{S} \right) \times 100$$

where a, b, c and d represent the number of seeds which germinated after 1, 2, 3 and 4 days from the start of germination, respectively. S represents the total number of germinated seeds.

1.3.3 Plant growth

Clover (*Trifolium repens* L., clone NC-S) and perennial ryegrass (*Lolium perenne* L.) plants were planted in monoculture or mixture in 36 dm^3 pots containing a fully characterized nutrient poor field soil. Plants were established in spring 1999 and were grown under field conditions until late-winter 2001 and hence, constituted long-established monoculture or mixed communities at the beginning of the oxygenized™ water study. From establishment, mesocosms were supplied with contrasting levels of nutrients, so establishing nutrient-rich and nutrient-poor plant communities. Mesocosms received either 3.3 g of 20/20/20 fertilizer (Chempack Products, Essex, UK) every month or 1.5 g of the same fertilizer only once in spring of each year.

On January 2002, 48 mesocosms were transferred and randomly positioned within 4 blocks on a glasshouse bench. The glasshouse maintained at a minimum night-time temperature of 18 ^{0}C. One month later, mesocosms were harvested (all vegetative material 5 cm above soil surface) and fertilized as above. Pots were watered as required with either oxygenized™ or tap water. At monthly intervals, the above-ground biomass was determined after plant material was dried to constant weight in an oven at 70 ^{0}C.

1.3.3 Statistical analyses

Statistical analyses were performed using SPSS (SPSS Inc., Chicago, Illinois, USA). Data for seed germination vigour were analysed using a *t*-test. Seed germination data were subjected to repeated measures analysis. Plant biomass data were subject to analysis of variance (ANOVA).

1.4 Results

1.4.1 Characterisation of water employed for experimental purposes

Dissolved oxygen (DO), temperature, pH, redox potential, conductivity, total hardness, total chlorine, free chlorine and total alkalinity levels of oxygenized™ and tap water immediately prior to experimental treatments are shown in the table below (Table 1.2).

Figure 1.2 shows the DO content and temperature during storage of oxygenized™ water for 63 h at 'room temperature'. DO content remained at ≈18 ppm for upto 11h and the temperature at ≈13^{0}C. DO content subsequently declined to 14-15 ppm as temperature, with the data revealing the expected inverse relationship between DO and temperature (Figure 1.3).

Table 1.2 Characterisation of oxygenized™ and tap water employed for experimentation

	Oxygenized™ water	*Tap water*
Dissolved oxygen (ppm)	18.2	10.8
Temperature (^{0}C)	13.0	12.0
PH	7.8	7.8
Redox potential (mV)	-380 to +50	+300
Conductivity (µS)	0	-
Hardness $CaCO_3$ (ppm)	50	200
Total chlorine (ppm)	2	0
Free chlorine (ppm)	3	0
Total alkalinity (ppm)	60	100

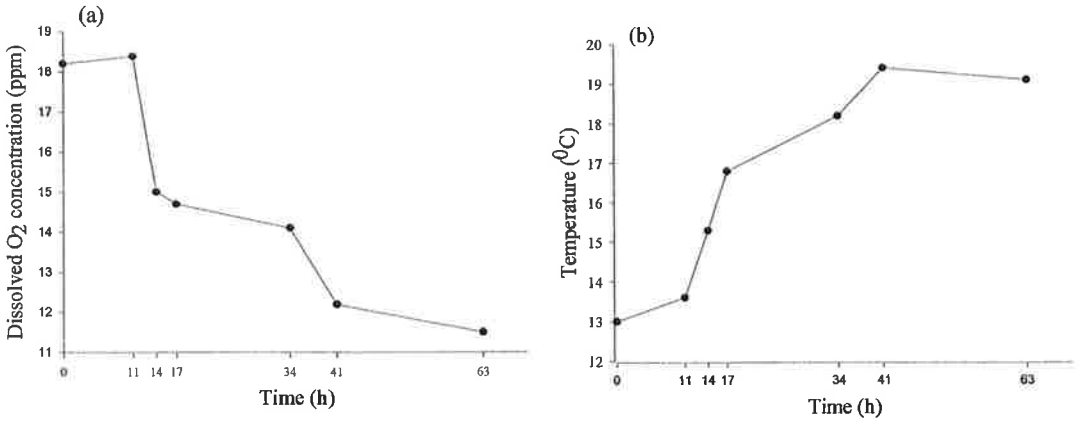

Figure 1.2 Changes in the (a) dissolved oxygen concentration (ppm), and (b) temperature (^{0}C) during storage of oxygenized^{TM} water for 63 h.

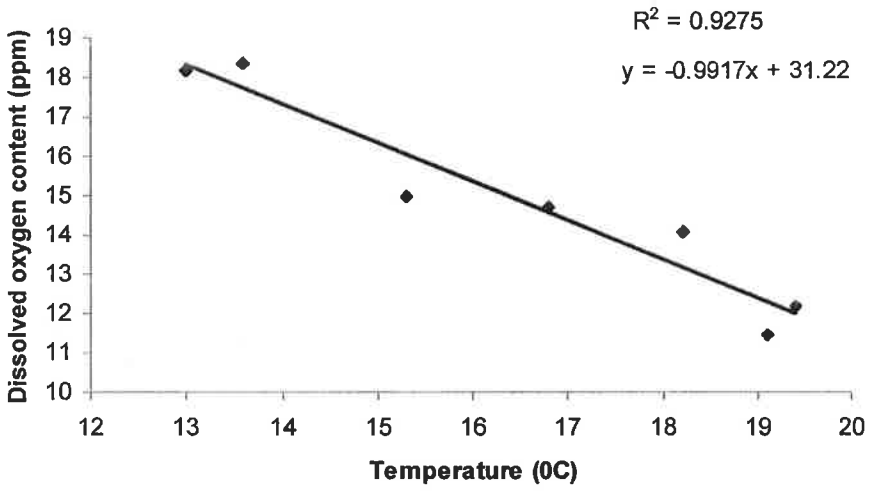

Figure 1.3 Relatioinship between DO and temperature during storage of oxygenized^{TM} water

1.4.2 Seed germination

- Oxygenized™ water improved germination vigour (a measure of the speed of germination) for seed of onion 'Bedfordshire champion' (P= 0.08) and 'Senshyu yellow' (P= 0.05), aubergine (P= 0.09), lettuce (P= 0.01) and *Ageratum houstonianum* (P= 0.05) (Figure 1.4). There was no significant effect of oxygenized™ water on seed vigour in cabbage (P= 0.11), carrot (P= 0.53), okra (P= 0.90), parsley (P= 0.23) and *Rudbeckia hirta* (P= 0.67).

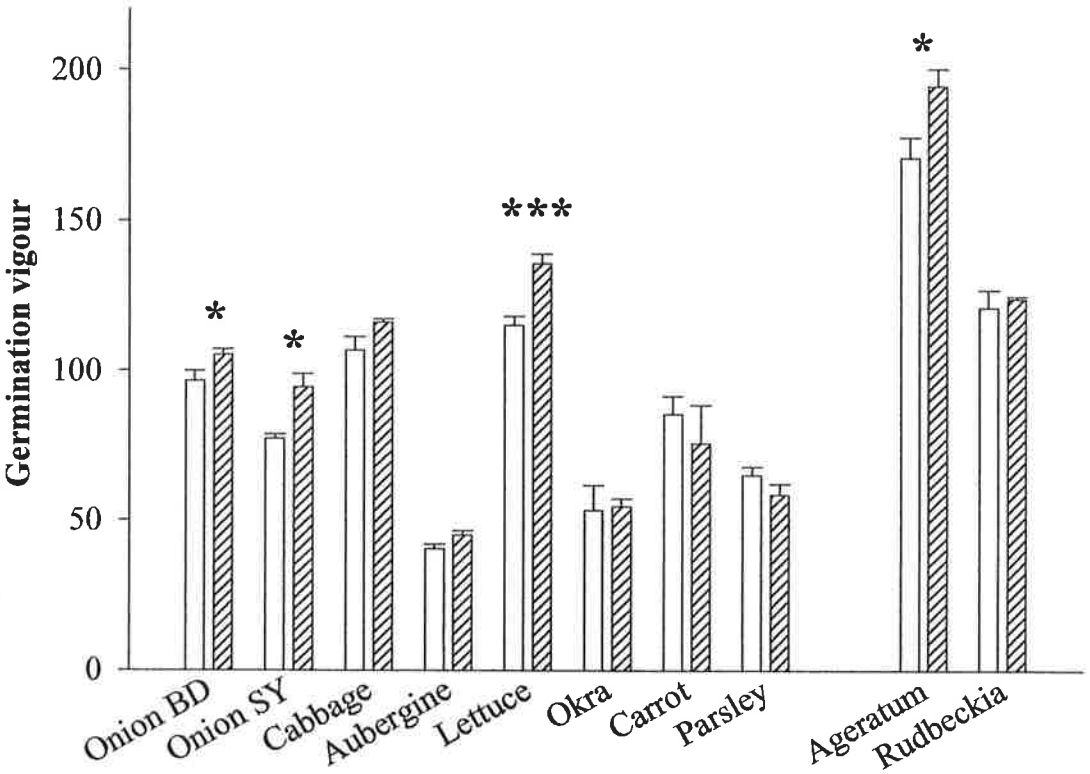

Figure 1.4 Effects of oxygenized™ water on seed germination vigour of onion var. Bedfordshire Champion and Senshyu yellow, cabbage var. Christmas drumhead, aubergine var. Honeymaker, lettuce var. Lollo Rosa, okra var. Clemsons spineless, carrot var. St. Valery, parsley var. Champion Moss curled 3, *Ageratum houstonianum* var. Blue Mink and *Rudbeckia hirta* L var. Marmalade.
Measurements were made on seeds germinated on filter paper in Petri dishes and supplied with tap (□) or oxygenized™ (▨) water. Each value represent the mean (±SE) of 3 independent petri dishes containing 50 seeds. Significant differences from tap water are indicated: * (P< 0.100), *** (P< 0.010).

- In addition, the rate of seed germination of onion var. Bedfordshire Champion ($P< 0.10$), cabbage ($P< 0.05$) and aubergine ($P< 0.05$) was improved by oxygenized™ water (Figure 1.5, Table 1.3). In onion var. Senshyu yellow and lettuce, the germination rate was increased by oxygenized™ water, but the effect was restricted to early stages of germination ($P< 0.05$) (Figure 1.6 and 1.7, Table 1.3). There was no significant ($P> 0.100$) effect of oxygenized™ water on the germination rate of okra, parsley, carrot, *Ageratum houst.* and *Rudbeckia hirta* seed (Figure 1.7, Table 1.3).

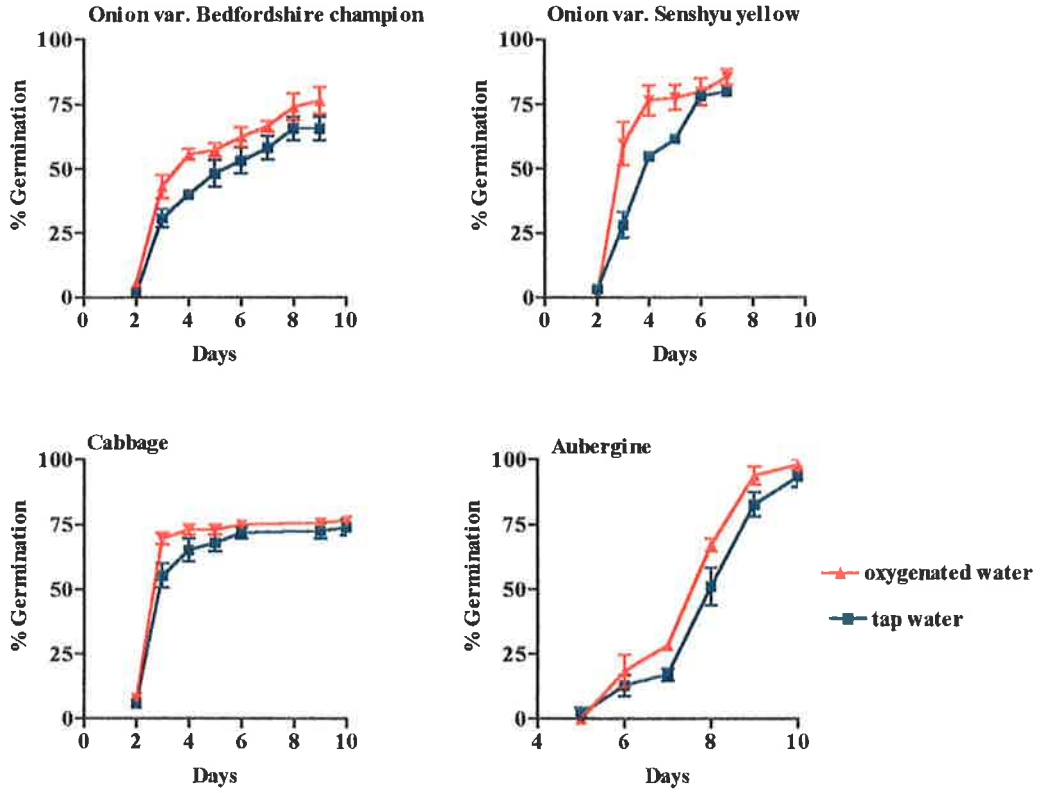

Figure 1.5 Effects of oxygenized™ water on the germination rate of onion vars. Bedfordshire Champion and Senshyu yellow, cabbage var. Christmas drumhead and aubergine var. Honeymaker.

Measurements were made on seeds treated with tap (■) or oxygenized™ (▲) water daily. Each value represents the mean (±SE) of 3 independent Petri dishes containing 50 seeds.

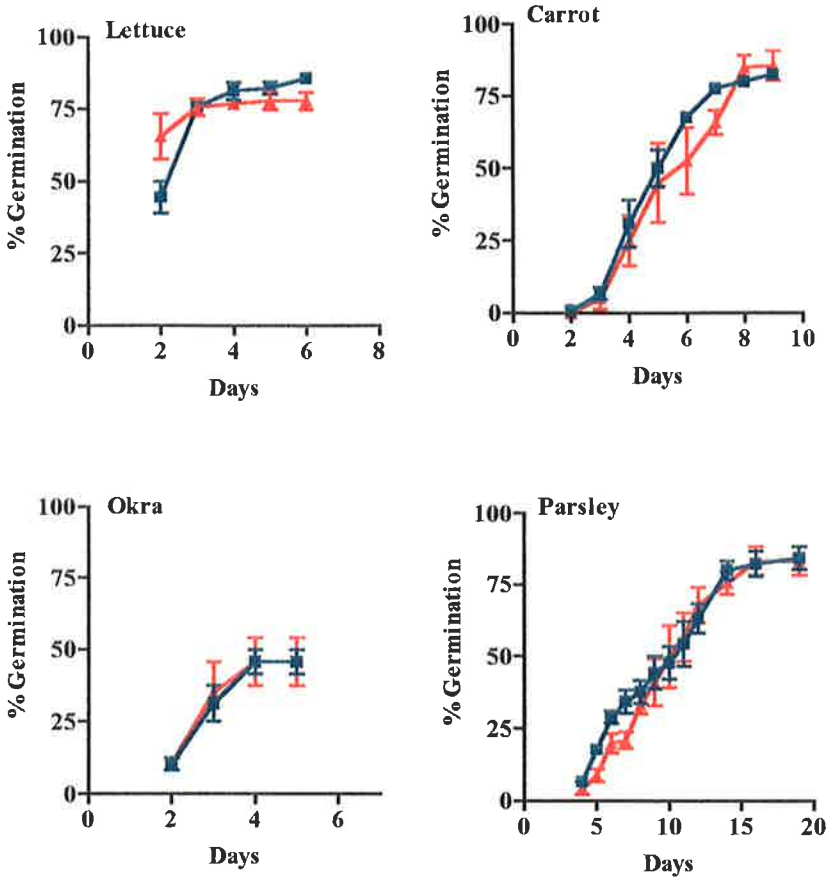

Figure 1.6 Effects of oxygenized™ water on the germination rate of lettuce var. Lollo Rosa, carrot var. St. Valery, okra var. Clemsons spineless and parsley var. Champion Moss curled 3.

Measurements were made on seeds treated with tap (■) or oxygenized™ (▲) water daily. Each value represents the mean (±SE) of 3 independent Petri dishes containing 50 seeds.

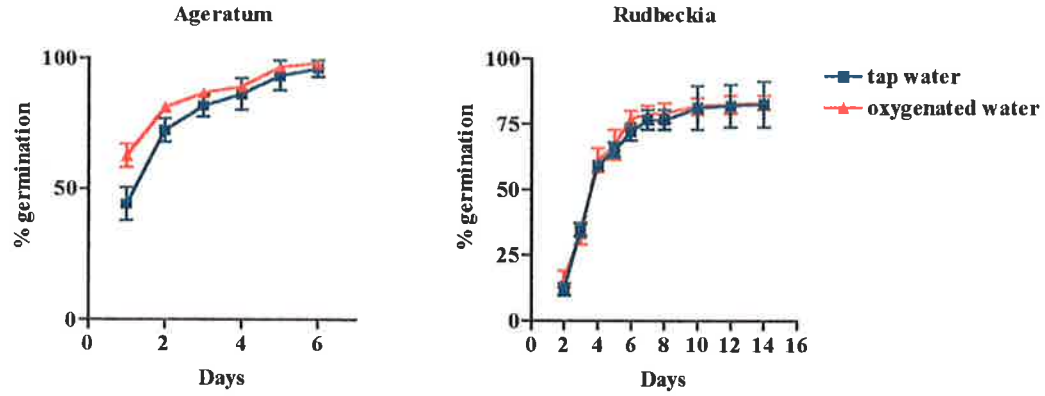

Figure 1.7 Effects of oxygenized™ water on the germination rate of *Ageratum houstonianum* var. Blue Mink and *Rudbeckia hirta* L var. Marmalade.

Measurements were made on seeds treated with tap (■) or oxygenized™ (▲) water daily. Each value represents the mean (±SE) of 3 independent Petri dishes containing 50 seeds.

Table 1.3 *P* values showing the effects of water quality, time and water quality x time interaction on the germination rate of vegetable seeds

	Water	*Time*	*Water x Time*
Onion var. Bedfordsh. Cham.	0.097	<0.001	0.790
Onion var. Senshyu Yellow	0.104	<0.001	0.009
Cabbage	0.003	<0.001	0.168
Aubergine	0.045	<0.001	0.394
Lettuce	0.970	<0.001	<0.001
Okra	0.907	<0.001	0.950
Parsley	0.561	<0.001	0.158
Carrot	0.387	<0.001	0.731
Ageratum houst.	0.392	<0.001	0.214
Rudbeckia hirta	0.898	<0.001	0.964

1.4.3 Plant growth

- Application of oxygenized™ water resulted in a significant (P= 0.07) increase in the biomass of clover and clover/grass mesocosms grown in nutrient-poor soil (Figure 1.8). There was no significant effect of oxygenized™ water on the biomass of clover and clover/grass mesocosms grown in nutrient-rich soil. However, there was no significant effect of oxygenized™ water on the biomass of grass grown in both nutrient-rich and nutrient-poor soil.

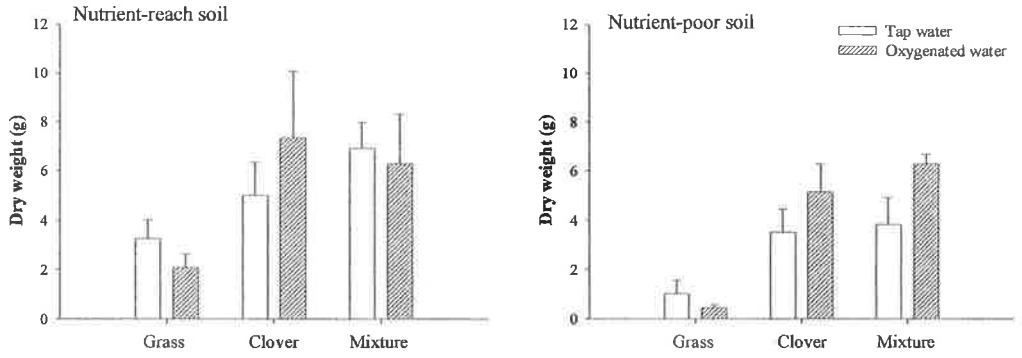

Figure 1.8 Effects of oxygenized™ water on biomass of grass, clover and gass/clover mesocosms supplied with tap (□) or oxygenized™ (▨) water. Each value represents the mean (±SE) of 4 independent mesocoms.

1.4.4 Crop yield

Seedlings raised from seed on oxygenized™ water have reached the repotting/pricking-out stage. First harvests have been made on some crops and the plant material is now drying in the oven. Provisional visual assessments would suggest that plants supplied with oxygenized™ water are substantially bigger/more advanced than plants fed tap water. Confirmation of visual indications is awaited.

1. 5 Summary

- Oxygenized™ water has the potential to improve seed germination. Application of oxygenized™ water enhanced seed vigour and/or germination rate in four out of six vegetable crops and one out of two ornamental crops screened to date.

- Utilising long-established grass/clover mesocosms to probe potential effects of oxygenized™ water on plants in nutrient-limited versus nutrient-rich environments, suggested that plants in nutrient-poor habitats may benefit more than those in nutrient-rich situations. Interestingly, the stimulation in growth resulting from the application of oxygenized™ water was particularly apparent in the legume component of grass/clover mesocosms. It remains to be established whether these trends will continue in subsequent harvests.

- Provisional indications suggest stimulations in growth/timing of some crops. Confirmation and quantification of provisional assessments is eagerly awaited *via* biomass data.

1.6 Comments on potential commercial applications of oxygenized™ water from the data gathered in weeks 1-8 of the project:

- The rate and uniformity of seed germination is an important factor underpinning successful crop establishment and thus oxygenized™ water may have potential applications for niche-markets in the horticultural sector (e.g. seedling crops e.g. supermarket herbs, mung beans etc. and/or high value difficult-to-germinate species e.g. many ornamentals, perennial shrubs, conifers etc.)

- If it proves to be the case that plants in nutrient poor habitats particularly benefit from the application of oxygenized™ water, this could open-up a number of interesting prospects e.g. potential applications in the recolonization of disturbed areas and mine sites and government-backed grassland diversification schemes. It is particularly interesting that the legume component appears to react so positively to oxygenized™ water application as legumes are particularly important pioneering species in recolonization programmes – since they open-up the existing vegetation and encourage colonization by other, targetted, species.

- It is too early to comment on wider applications in the horticultural sector in terms of effects on crop yield – but early indications are positive.

1.7 Ongoing work during the period April-July 2002

- Investigate the effects of oxygenized^{TM} water on seedling growth and crop yield – focussing on lettuce, carrot, onions, tomatoes and parsley – given the remaining time available to conduct the contracted work programme
- Extend studies on rate and speed of germination for species difficult to germinate and of high economic value, potentially opening-up novel commercial avenues.

1.8 Suggested priorities for future work to underpin potential commercial applications for oxygenized^{TM} water in the horticultural sector

- Investigate the **basis of the stimulation in plant growth** resulting from the application of oxygenized^{TM} water through the characterization of shifts in photosynthetic capacity, root/shoot respiration, leaf/fruit nutrient status and soluble carbohydrate status (plus potentially a full characterization of produce quality-related variables ?). The particularly strong reaction of the legume component of grass/clover mesocosms is intriguing since it it known that in such species, poor nutrient supply (Van der Werf and Lambers, 1996) and symbiotic mycorrhizal fungi (Lambers *et al.,* 1998) increase the fraction of carbohydrates used for respiration so it is possible that oxygenized^{TM} water overcomes DO limitations in such circumstances.

- Investigate whether oxygenized^{TM} water can be used as a medium for **hydropriming**. Seed priming (by letting seeds imbibe pre-sowing) is widely used to enhance seed performance with respect to rate and uniformity of germination (e.g. Capron *et al.,* 2000) and hydropriming is a simple priming method (Fujikura *et al.,* 1993). The range of oxygen concentrations that are effective in priming have been shown to be the same with those that allow germination to occur (beetroot, Capron *et al.,* 2000; tomato, Özbingöl, 1998).

- Investigate the potential of oxygenized water to overcome the oxidative stress imposed on plants in their natural/glasshouse surroundings by a variety of environmental stresses. The majority of environmental stresses damage plants *via* the production of reactive oxygen species. When the titre of these species exceeds the palnts natural capacity to defend itself, then damage and eventually death can occur. The reactive oxygen species generated by all manner of environmental stresses are the cause of substantial reductions in crop yield in all farming/horticultural situations. Antioxidants protect tissues from the effects of reactive oxygen species or free radicals. Recent studies by Hanaoka (2001) and Shirahata *et al.* (1997) have shown that reduced water – a quality attribute exhibited by our oxygenized™ water - has antioxidant properties. If oxygenized™ water proves to have similar **antioxidant properties**, this will open an avenue for exploitation in improving the resistance of plants to environmental stresses responsible for several billion dollars crop loss in the USA alone.

- Investigate the potential of oxygenized™ water for **arable weed control.** Increased ambient oxygen concentration has been found to improve seed germination in weed species such as *Sinapis arvense*, *Eriochloa villosa, Rumex crispus* and *Bidens pilosa* (Mayer and Poljakoff-Mayber, 1977). Should oxygenized™ water prove to be an effective agent in breaking annual weed seed dormancy, then it might give rise to new management/eradication systems for these weeds through pre-plant applications to force the seed bank through in agricultural circumstances.

- **Commercial-scale trials** are necessary for bridging the gap from laboratory-based findings to commercial-scale benefits. Suggested that these trials take place with grower-base with whom we already have links.

- Examine potential for use in **organic sector** through liason with colleagues who are movers and shakers in this area , affiliated to the Tesco Institute for Organic Agriculture (based at Newcastle University).

- Provide directed **market analysis** by commissioning experts at RTC North

Part 2: Decontamination of freshly-harvested produce

Raveenia Roberts, Ian Singleton and Jeremy Barnes

2.1 Introduction

As much as 30% of fresh produce can be lost through microbial spoilage during post-harvest handling, transport, storage and packing (Beuchat, 1992). Indeed, freshly-harvested fruit and vegetables commonly have on them anywhere from 10^3-10^9 micro-organisms per gram. Given an initial microflora of 10^3-10^5 microbes per gram, spoilage generally occurs within 10-14 days at a temperature of 22°C (Smilanick *et al.* 1995). Research has clearly demonstrated that the lower the microbial infection load, then the longer the expected storage/shelf-life of the produce – hence the commercially-adopted tactic of refrigerating produce in storage/transit and adding a variety of sterilants to wash-water. Recent EU council regulations (e.g. EEC 2092/91 and Biocides Directive 98/8/EC) are moving toward a ban on the use of some of the most commonly adopted sanitisers such as hypochlorite/chlorine dioxide. This poses a serious dilemma for growers/producers and there is considerable commercial interest in potential alternatives – particularly for use in the organic foods/produce sector.

The situation yields a timely window of commercial opportunity if oxygenized™ water is proven to be effective in discouraging the development of microbes that cause a variety of post-harvest disorders (e.g. *Botrytis cinerea*, *Mucor pyriformis*, *Rhizopus nigricans*, *Penicillium* spp., *Alternaria* spp. *Pythium* spp., *Cladosporium* spp., *Sclerotinia* spp. and *Erwinia cartovora*).

2.2 Aims and Objectives

- Quantify the inactivation of surface-borne pathogens by oxygenized™ water on a variety of crops under carefully monitored and reproducible conditions.
- To determine the effects of oxygenized™ water treatments on spore germination of targeted fungal pathogens
- To investigate the effects of oxygenized™ water on the development of *Botrytis cinerea* (grey mould) on tomato fruit (*Lycopersicon esculentum*)
- To explore the up-regulation of endogenous antimicrobial defences in fruit through the application of oxygenized™ water

2.3 Materials and Methods

2.3.1 Efficacy of oxygenized™ water for the surface disinfection of fresh produce

Six replicates of each commodity (see Table 2.1), except lettuce (100 g or 4 leaves) and grape (60 fruit), were washed in running oxygenized™ water or tap water (0.5 gallon min^{-1}) for 15, 30, or 60 min. After treatment, replicates were washed in 200 ml sterile Phosphate buffered saline (PBS, pH 7.2, Gibco, Life Technologies, Paisley, UK) for 10 min, and 1 ml was removed and serially diluted in PBS. For each treatment, 0.1 ml of diluent was plated, in triplicate, onto nutrient agar (NA) amended with Nystatin (for bacteria) or rose bengal chloramphenicol agar (RBC, for fungi), and incubated for 48 h or 5 d, respectively.

Table 2.1 Commodities used for oxygenized™ water treatments

Commodity	Surface Area (± SE)
Apple (*Malus domestica* cv. Royal gala)	185.38 ± 11.11
Carrot (*Daucus carota)*	131.18 ± 7.43
Grape (*Vitis vinifera* cv. Thompson seedless)	14.93 ± .04
Lettuce (*Lactuca sativa* cv. Iceberg)	3052.67 ± 232.60
Pear (*Pyrus communis* cv.William)	78.73 ± 4.00
Tomato (*Lycopersicon esculentum)*	96.21 ± 0.82

2.3.2 Impacts of oxygenized™ water on germination of targeted fungal pathogen spores

Spores of *Botrytis cinerea* (grey mould)*; Penicillium* spp. (blue mould); *Cladosporium* spp. (green mould); *Helminthosporium solani* (silver scurf) and *Colletotrichum coccodes* (black dot) were washed from 7-10-day-old cultures raised on potato dextrose agar (PDA) and suspended in sterile distilled water. 0.2 ml of this stock suspension was added to sterile glass tubes containing 1.8 ml oxygenized™ water or tap water to achieve a final concentration of 2×10^5 spores ml^{-1}. After contact for 0 (control, tap), 15 and 30s, 1,2, 5, 10, 30, 60 min, a sample was removed, plated onto PDA and incubated at 20°C in the dark for 48 h. Percentage spore germination was calculated from the mean germination of 50 spores. Spores were considered to have germinated if the length of the germ tube was at least half the diameter of the spore.

2.3.3 To investigate the effects of oxygenized™ water on the development of *Botrytis cinerea* (grey mould) on tomato fruit

Tomato fruit were pierced with a sterile scalpel to produce a wound 3 mm deep and 2 mm wide. Three wounds were made on each fruit. Spores were washed from 7-day-old PDA cultures of *Botrytis cinerea* with 5 ml of sterile distilled water and suspended at a concentration adjusted to 2 x 10^5 spores ml^{-1}. Thirty microliters (30 µl) of the suspension was placed into each wound. Inoculated fruit were incubated at room temperature for 4 h to allow the adhesion of spores prior to wash treatment.

Tomatoes were either inoculated with *Botrytis cinerea* spores and not washed (unwashed control), inoculated with *Botrytis cinerea* spores and then washed with either tap or oxygenized™ water for 15, 30, or 60 min. Fruit were dried, then maintained at 13°C in a controlled environment room. Fungal lesion area was measured over a period of 7 days.

2.3.4 Explore the up-regulation of endogenous antimicrobial defences in produce triggered by the application of oxygenized™ water

Fruit were washed in oxygenized™ or tap water for 1 h, dried and wounded as described in section 2.3.3. Mycelial plugs (2.5 mm diameter) were removed from the advancing margins of 2-day-old *Botrytis cinerea* PDA cultures and placed inside each wound. Inoculated tomatoes were incubated at 13°C and lesion area development was measured over a period of 7 days.

2.2.5 Statistical analyses

Data were analysed using SPSS (SPSS Inc., Chicago, USA). Individual means were compared using a t-test. Data involving repeated measurements on the same samples were subjected to repeated measures analysis of variance (ANOVA).

2.4 Results

2.4.1 Efficacy of oxygenized™ water for the surface disinfection of fresh produce

The range and numbers of microbes present on fresh fruit and vegetable surfaces at harvest are highly variable, and often reflect the microflora present in the field at the time of harvest (DeRoever, 1998). Moreover, the initial microbial load differs depending on the type of produce and climatic and agricultural determinants such as geographical location, weather conditions, irrigation systems and pre-harvest to post-harvest practices (Beuchat and Rhu, 1997; Brackett, 1999).

Figure 2.1 illustrates the impacts of oxygenized™ water on the total fungal population retained on the surface of various commodities following washing for 15, 30 min or 1 h.

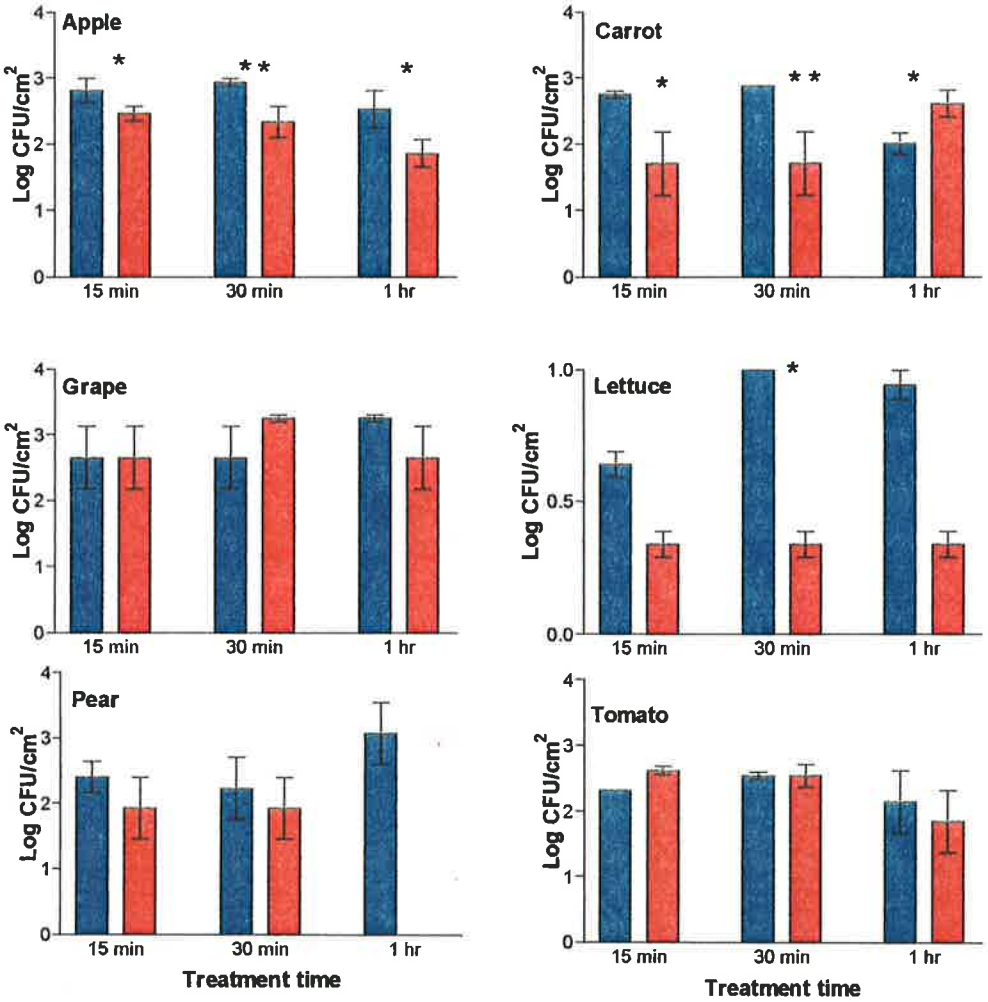

Figure 2.1. Fungal counts (log $_{10}$ CFU/cm^2) on produce after washing in oxygenized™ water or tap water for 15, 30 or 60 min. Diluent was inoculated onto rose bengal chloramphenicol agar (RBC) and incubated for 5 days at 22°C. Values are means (± SE) of 3 replicate plates. Tap water ( ) and oxygenized water™ ( ).

- Oxygenized ™ water significantly reduced fungal counts in 3 out of the 6 commodities tested compared with tap water
- Overall, no significant treatment*treatment time interaction

Figure 2.2 illustrates the impacts of oxygenized™ water on the total bacterial population retained on the surface of various commodities following washing for 15, 30 min or 1 h.

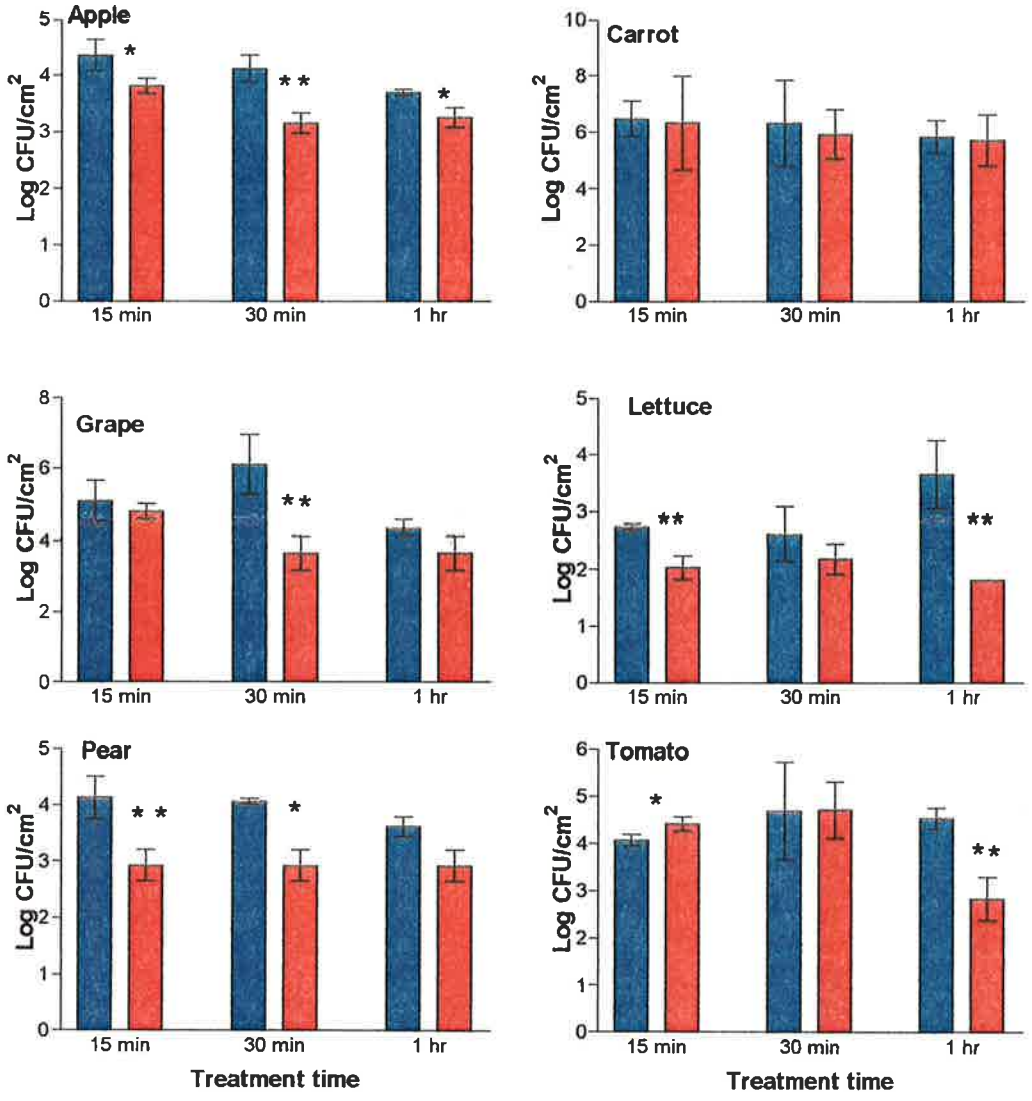

Figure 2.2. Bacterial counts (log $_{10}$ CFU/cm^2) on produce after washing in oxygenized™ water or tap water for 15, 30 or 60 min. Diluent was inoculated onto nutrient agar (NA) and incubated for 2 days at 22°C. Values are means (± SE) of 3 replicate plates. Tap water ( ) and oxygenized water™ ( ).

- Oxygenized™ water significantly reduced bacterial load (~2 $\log_{10}$ cfu/cm^2 reduction) on all produce, except carrots (5/6).
- Bacterial load reduction varied between commodity

2.4.2 Effects of oxygenized™ water on germination of targeted fungal pathogens spores

The pathogen spores investigated, all constitute sources of considerable postharvest spoilage. Postharvest pathogens account for considerable economic losses worldwide (e.g. the British Potato Council (1995) estimated potato losses due to *Colletotrichum coccodes* and *H. solani* to be in excess of £5 million, alone). Spores represent a vital phase in the life cycle of filamentous fungi. Spores not only provide a mechanism for survival in variable environments, but constitute the reproductive structures that are more resistant than their vegetative forms to most types of physical and chemical stresses.

Figure 2.3 illustrates the almost immediate effects of oxygenized™ water on the spores of two important potato pathogens resulting in skin blemishes on salad and ware potatoes. Interestingly, fungicides are available to control *Helminthosporium*, but *Colletotrichum* is notoriously much harder to control. Oxygenized™ water for some as yet unknown reason seemed particularly effective in inhibiting the germination of *Colletotrichum* spores, though it was also effective against *Helminthosporium*.

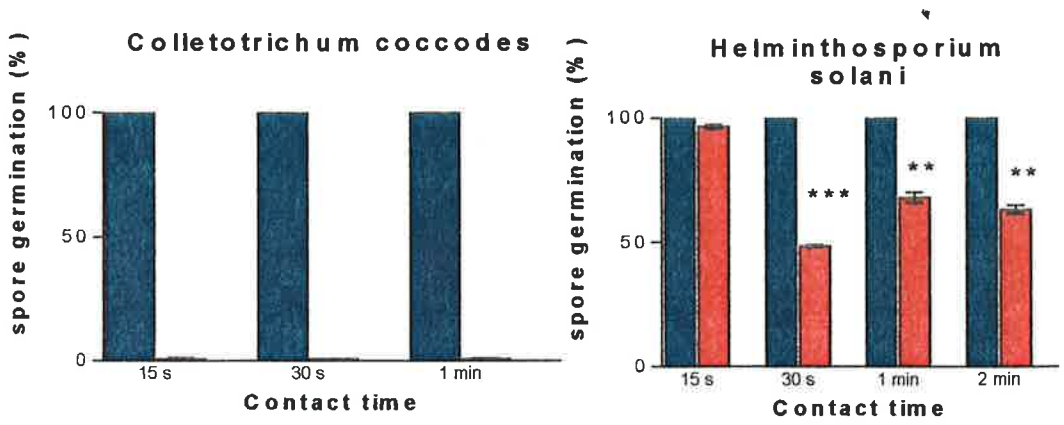

Figure 2.3 Percentage germination for *Colletotrichum coccodes* (black dot of potato) and *Helminthosporium solani* (silver scurf of potato) spores after 15 s to 2 min immersion in oxygenized™ water or tap water. A 0.1 ml spore suspension was removed after 15 and 30 s and 1 or 2 min contact time in oxygenized™ water or tap water and plated onto PDA and incubated at 20°C in the dark for 48 h. Bars represent the mean (± SE) of 3 replicate plates. Tap water (■) and oxygenized water™ (■).

- In *Colletotrichum coccodes* oxygenized™ water was effective in preventing spore germination in <15 s contact time ($P<0.001$)
- In *Helminthosporium solani* significant reduction ($P<0.001$) with oxygenized™ water >15 s contact time.

Figure 2.4 illustrates the effects of oxygenized™ water on the spores of three other common and economically-crippling pathogens. Longer contact times than were observed for the two potato pathogens were found to be required to result in inhibition of spore germination, but for all pathogens studied oxygenized™ water resulted in pronounced and effective spore inactivation.

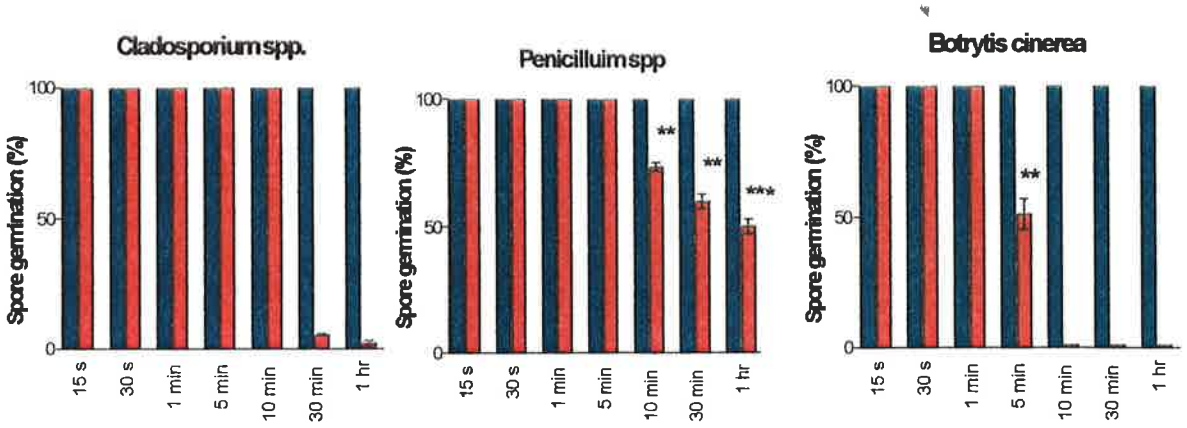

Figure 2.4 Percentage germination for *Cladosporium* spp., *Penicillium* spp., and *Botrytis cinerea* spores after 15 s to 60 min immersion in oxygenized™ water or tap water. A 0.1 ml spore suspension was removed after 15 and 30 s and 1 or 2 min contact time in oxygenized™ water or tap water and plated onto PDA and incubated at 20°C in the dark for 48 h. Bars represent the mean (± SE) of 3 replicate plates. Tap water ( ) and oxygenized water™ ( ).

2.4.3 Effects of oxygenized™ water on the development of *Botrytis cinerea* (grey mould) on tomato fruit

Figure 2.5 shows the pronounced fungistatic action of oxygenized™ water on the development of grey mould on tomato.

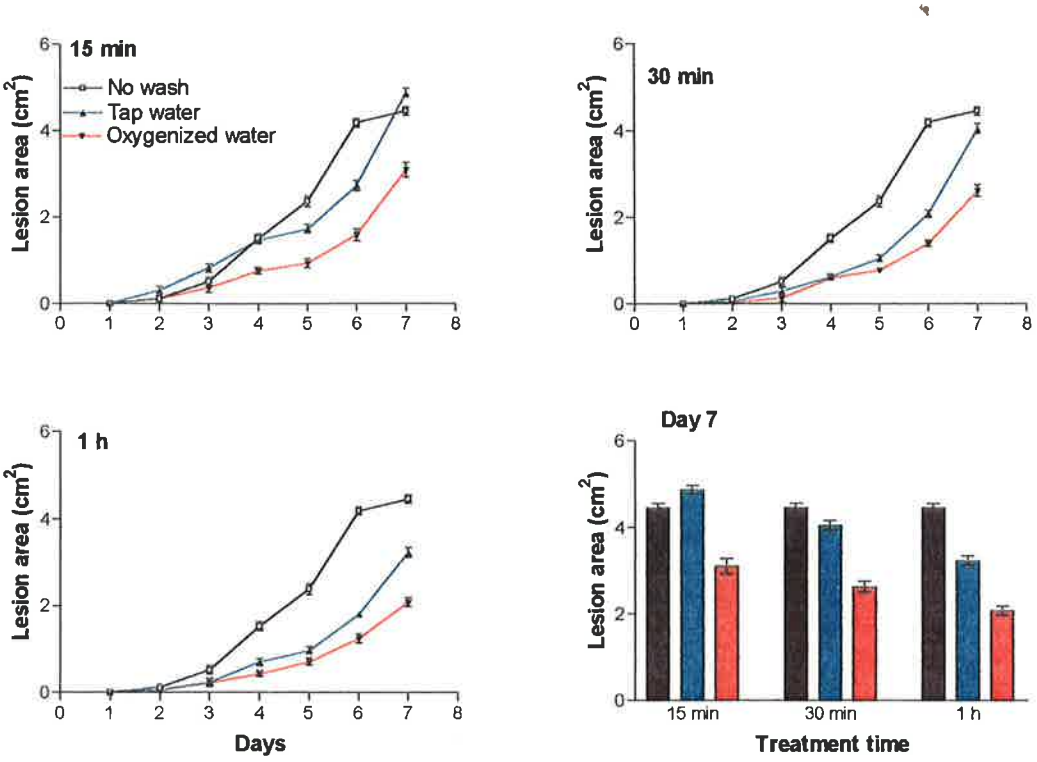

Figure 2.5 Effects of oxygenized™ water treatments on lesion area development on tomato fruit. Fruit were inoculated with 30 µl of a 2 x 10^5 *Botrytis cinerea* spore suspension, allowed 4 h incubation time prior to being washed for 15, 30 or 60 min in oxygenized ™ water or tap water. Inoculated fruit were incubated at 13°C and lesion development was recorded over 7 days. Values are the mean (± SE) of 9 replicate lesions.

2.4.4 Explore the up-regulation of endogenous antimicrobial defences in produce through the application of oxygenized™ water

Figure 2.6 shows that pre-treatment with oxygenized™ water resulted in a small but significant ($P < 0.05$) reduction in fungal development – in fruit inoculated shortly after a 1h immersion in oxygenized™ versus tap water

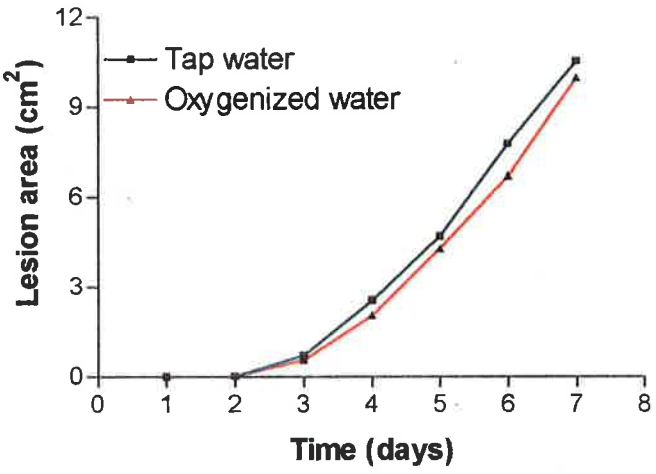

Figure 2.5 Effect of oxygenized™ water on the up-regulation of endogenous antimicrobial defences in tomato fruit. Fruit were washed for 1 h in oxygenized™ water or tap water, dried and inoculated with 2.5 mm *Botrytis cinerea* mycelial plugs. Inoculated fruit were incubated at 13°C and lesion area development was recorded over a period of 7 days. Values are the means (± SE) of 9 replicates.

2.5 Summary

- Washing produce in oxygenized™ water can significantly reduce microbial load compared to washing in tap water. However, the efficacy of the treatment appears to be commodity-dependent
- Oxygenized™ water inhibits the germination of spores of common fungal pathogens – though optimal treatment times seem to vary between species (Treatment* contact time interaction, $P < 0.001$)

- Oxygenized ™ water was twice as effective ($P<0.01$) as tap water in controlling grey mould development on tomato fruit, with the extent of the effect dependent on immersion time (treatment* treatment time interaction, $P< 0.001$)
- Oxygenized ™ water treatment appeared to provide a double kickback in that pretreatment reduced subsequent fungal development. Presumably this resulted from the up-regulation of endogenous antimicrobial defences in tomato fruit as well as direct effects on the microbial load.

2.6 Comments on potential commercial applications of oxygenized™ water from the data gathered in weeks 1-8 of the project:

- Strong suggestion that oxygenized™ water may provide a means of reducing microbial spoilage of commodities through its antimicrobial action. Providing commercial opportunities to harness the benefits afforded by oxygenized™ water for the washing/decontamination of fresh produce pre-store and/or pre-pack.

2.7 Ongoing work

- Repeat surface washes on a larger range of produce of varying surface morphology,
- Focus on a range of targeted pathogens present both fresh and pre-washed commodities (e.g. potatoes and salads)
- Establish optimum contact times for surface disinfection for specific commodities
- Manipulate redox potential to enhance its biocidal properties
- Test impacts of optimized treatments on the commodity

2.8 Suggested priorities for future work to underpin potential commercial applications for oxygenized™ water in the horticultural sector

- Why effective in decontamination of **some commodities but not others** ? are the differences observed in the efficacy of oxygenized™ water due to (i) variations in surface morphology (ii) variations between pathogens in their 'resistance' to

oxygenized™ water or (iii) variations in treatment application conditions – would additional agitation improve efficacy of treatments ?

- **Mode of action**. How does oxygenized™ water exert it's antimicrobial effects ? what are the effects on spores ? bacteria (differences in 'sensitivity' between gram negative and gram positive strains?) what are the effects on fungal mycelia ? what effect does oxygenized water exert to reduce post-treatment fungal development ? Undertake exposure-response studies in relation to DO concentration and its effectivity in pathogen control
- Investigate **contact times** with a view to harnessing oxygenized™ water for contrasting washing processes (e.g. spray applications, dips or washes)
- Investigate additional applications in **hydroponic culture** and/or **irrigation systems** e.g. inter-crop decontamination of beds, *in situ* pathogen control/yield stimulation, potato production.
- There is growing concern over the levels of **human pathogens** (e.g *Campylobacter sp., Cryptosporidium, Listeria, Coliforms, Salmonella, Giardia, Clostridium*) that contaminate the surface of many salad crops in particular (e.g. herbs and lettuce) and an urgent need to examine new and effective treatments to combat the survival/growth of these diseases on the surface of fruit and vegetables
- **Commercial-scale trials** are necessary for bridging the gap from laboratory-based findings to commercial-scale benefits. Suggested that these trials take place with grower-base with whom we already have links.
- Examine potential for use in **organic sector** through liason with colleagues who are movers and shakers in this area , affiliated to the Tesco Institute for Organic Agriculture (based at Newcastle University).
- Provide directed **market analysis** some of which has already been obtained for another purpose through experts at RTC North