Project Title	The use of carbon dioxide as an anaesthetic/muscle relaxant to aid removal of invertebrate contaminants in salad crops
Project number:	FV 295
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Location of project:	SAC, West Mains Road, Edinburgh
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The results and conclusions in this report are based on a series of experiments conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with 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 interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

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

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Grower Summary

Headline

- Contamination of mechanically harvested vegetables with insects and other invertebrates can be reduced by exploiting their behavioural response to disturbance and briefly exposing them to carbon dioxide.
- The concentration and length of exposure to elevated carbon dioxide reduces the force needed to dislodge invertebrate contaminants from salad leaves.
- This knowledge opens the way to developing an engineering solution utilising exposure to elevated carbon dioxide as a means to enhance the removal of contaminants from vegetables.

Background and expected deliverables

Insect and slug contamination of harvested vegetable crops is a serious concern when mechanically harvesting field vegetable crops. Penalties to vegetable suppliers for contamination are high, and there is a recognised need by the industry to improve on the current methods of contaminant removal.

What is evaluated in this study is the development of a process that can dislodge live contaminants during or after harvest. To enable the design of an improved solution, information on the threshold force required to remove invertebrate contaminants is required. The use of carbon dioxide as an anaesthetic/muscle relaxant is evaluated to determine the reduction in force needed to subsequently dislodge invertebrate contaminants.

The exploitation of a behavioural mechanism called thanatosis in invertebrates, where they ‘play dead’ when threatened, is assessed for the main contaminant species found in baby salad crops.

An engineering solution to invertebrate contamination could be more cost effectively implemented at field scale if it is designed and constructed using data on thanatosis, carbon dioxide concentrations, length of exposure to elevated carbon dioxide and the mechanical force to dislodge invertebrates, all of which will be derived from this project.

The expected deliverables from this study are:

- An understanding of the behavioural response of invertebrates to mechanical disturbance on baby leaf salads
- Quantification of the forces necessary to mechanically dislodge invertebrates from baby leaf salads
- Information on whether the use of carbon dioxide as an invertebrate anaesthetic/muscle relaxant can enhance dislodging of invertebrates from baby leaf salads
- Provide data for the development of an engineering solution to remove invertebrate contaminants from salad crops

Summary of the project and main conclusions

Invertebrate behaviour on being disturbed

A range of common contaminants were studied to see if they ‘played dead’ (becoming immobile for several seconds and consequently falling off a leaf) in response to mechanical stimulation.

All invertebrates tested exhibited some degree of 'playing dead' on being mechanically disturbed. Ladybirds, ground beetles and larger caterpillars were most likely to lose grip and ‘play dead’ when disturbed. Aphids and slugs were less so, depending on whether aphids were feeding, and the size of the slug.

Determining the force needed to remove invertebrates from leaves
A range of forces were applied to leaves which had invertebrates on them, to determine the minimum force necessary to dislodge the invertebrate. The apparatus used for measuring the force necessary to dislodge an invertebrate from a leaf is shown below.

Figure 1: Drop apparatus used to measure the force necessary to dislodge an invertebrate from a leaf

Five settings were used which relate to the height of the ‘drop’ of the arm holding a plastic container which inside had a metal plate to which the leaf is attached via sticky tape. The accelerative force needed to dislodge an invertebrate was measured for several settings using an accelerometer. Accelerative force (g) ranged from Force setting 1 (480g) to Force setting 5 (51g).

Table 1: The percentage of invertebrates dislodged from a leaf when subjected to several accelerative forces using the drop apparatus:

Percentage of invertebrates dislodged from the leaf

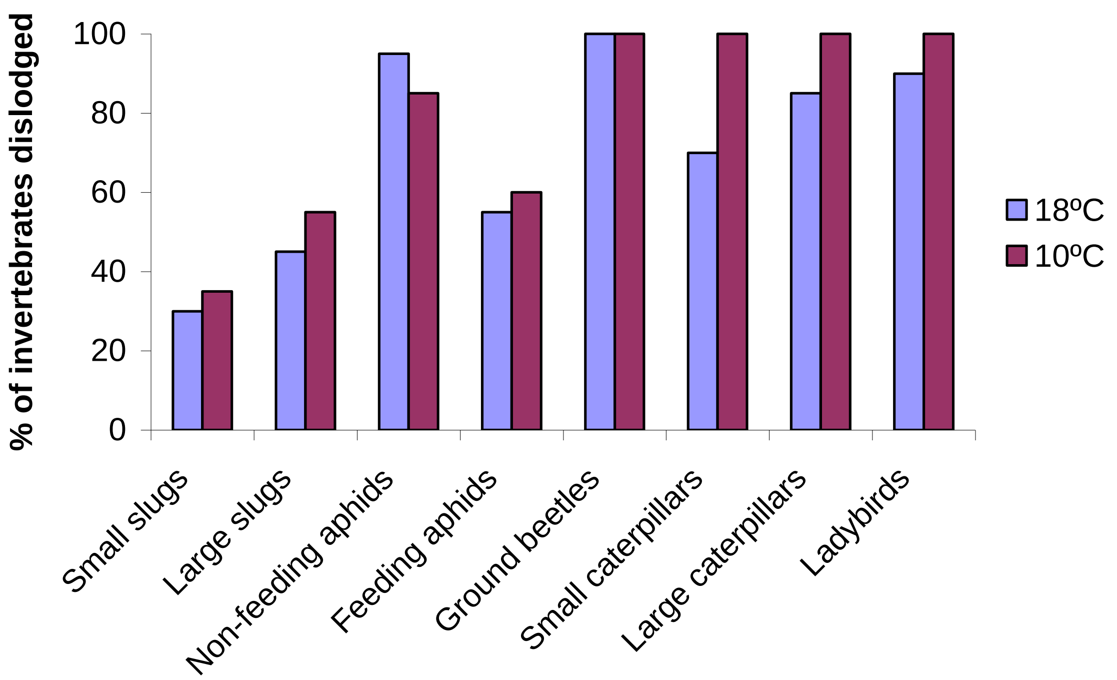
Force setting* (g)	Lady-birds	Caterpillars		Aphids		Ground beetles	Slugs	
		Small	*Large*	*Not feeding*	*Feeding*		*Small*	*Large*
1 - 480g	100	100	100	100	90	100	95	100
2 - 331g	100	100	100	100	80	100	80	100
3 - 181g	90	70	85	95	55	100	30	45
4 - 72g	50	30	50	35	10	30	10	15
5 - 51g	10	15	15	20	5	5	5	5

In terms of force applied to the leaf, feeding aphids and small slugs required the greatest (480g) to achieve the greatest level of removal, and ground beetles the least (181g).

Effect of temperature on the force needed to remove invertebrates

When the same accelerative force is applied to the leaf, lower temperatures (10ºC) tended to increase the numbers of invertebrates removed from leaves compared to 18ºC.

Figure 2: Effect of temperature on dislodging of invertebrates from salad leaves when the same force is applied to the leaf



Effect of carbon dioxide concentration and duration of exposure on the force needed to remove invertebrates

Invertebrates were exposed to a range of concentrations of carbon dioxide and a range of exposure times to assess whether the force needed to remove them from leaves was reduced.

Elevated carbon dioxide concentration and length of exposure significantly reduced the applied force required to remove invertebrates from leaves.

Table 2: Minimum applied Force (g), carbon dioxide (CO_2) concentration (%) and duration of exposure (seconds) to achieve 100% removal of invertebrates from leaves:

	Force (g) required to dislodge 100% of invertebrates from leaf		

Invertebrate	At ambient CO_2	At elevated CO_2	Optimal CO_2 concentration* %	Duration of exposure to CO_2 (s)
Small slugs	480	181	10	30
Large slugs	331	181	25	10
Non-feeding aphids	331	181	5	10
Feeding aphids	480	181	25	30
Ground beetles	181	72	25	30
Small caterpillars	331	181	25	10
Large caterpillars	331	72	25	10
Ladybirds	331	72	10	10

* Optimal CO_2 concentration refers to the concentration that led to 100% dislodging of invertebrates from the leaf.

In tests carried out in a wind tunnel, tunnel speeds of 10-20 ms^{-1} were required to dislodge beetles, aphids and caterpillars at ambient carbon dioxide levels. Beetles exposed to air + 9% carbon dioxide for 15 seconds were dislodged at tunnel speeds of 3-3.5 ms^{-1}, whereas beetles exposed to air only at tunnel speeds of 3-3.5 $ms-^{1}$ were not dislodged.

Financial benefits

The contamination of processed salads by invertebrates such as caterpillars, slugs, aphids, ladybirds, beetles and other species is unacceptable to the retailer and consumer. It is estimated that there are 4 customer/retailer complaints per 100,000 units for outdoor salad crops. Actual contamination incidence is likely to be 10 times this figure, as it is estimated that only 10% of customers actually complain. The presence of contaminants in produce can result in financial penalties to the grower or processor from retailers, and threatens the relationship between the grower and the retailer if contamination cannot be minimised. Salad growers estimate that a tenfold reduction in complaints annually may be worth as much as £500,000 to the industry. As many of these contaminants are transient, are often not pests of the crop and are simply caught up in the harvest, the use of insecticides for their removable is not justifiable to processors, retailers, or the consumer.
By developing a solution to contamination of salads, particularly baby-leaf salads, customer satisfaction will be achieved with a reduction in the complaints received and subsequent financial penalties.

As carbon dioxide is approved as a commodity substance by the Pesticides Safety Directorate for use as an insecticide, acaricide and rodenticide in food storage, an extension of use as an anaesthetic/muscle relaxant will not require any additional regulatory hurdles to be to be surmounted.

The approach outlined in this project does not aim to kill insect contaminants but simply to dislodge them from plants. Consequently, this method will cause minimal harm to non-pest insects such as ladybirds and ground beetles and will be favoured from an environmental and retailer/consumer viewpoint.

Action points for growers

- Contamination of salad crops by invertebrates such as aphids, beetles, and slugs can potentially be reduced through the use of carbon dioxide.

- Carbon dioxide acts as an anaesthetic/muscle relaxant to loosen the grip of invertebrates on the leaves, allowing them to be removed through mechanical stimulation.
- Some contaminants such as feeding aphids and small slugs are more difficult to remove than others.
- Salad growers will be consulted to determine whether a larger project to develop a prototype system utilising carbon dioxide either mounted on the harvester or during the washing/packing of salads is desirable.

Science Section

Introduction

Contamination of mechanically harvested vegetables, particularly processed salads, is a major problem to the industry (Lole, 2002; Pearson, 2004; Tatchell, 2005). The contamination of processed salads by invertebrates such as caterpillars, slugs, aphids, ladybirds, beetles and other species is unacceptable to the retailer and the consumer. It is estimated that there are 4 customer/retailer complaints per 100,000 units for outdoor salad crops. Actual contamination incidence is likely to be 10 times this figure, as it is estimated that only 10% of customers actually complain (Pearson, 2004). The presence of contaminants in produce can result in financial penalties to the grower or processor from retailers, and threatens the relationship between the grower and the retailer if contamination cannot be minimised. Salad growers estimate that a tenfold reduction in complaints annually may be worth as much as £500,000 to the industry. As many of these contaminants are transient, are often not pests of the crop and are simply caught up in the harvest, the use of insecticides for their removable is not justifiable to processors, retailers, or the consumer.

This problem exists worldwide - contamination of harvested food exists in all edible crop sectors. The HDC recently commissioned two reviews of methods that are potentially available for the removal of insects from outdoor salad crops in order to identify potential solutions to this problem (Pearson, 2004; Tatchell, 2005). These reviews addressed the potential for physical removal of contaminants from outdoor salads and at a HDC workshop held in 2005, recommended that invertebrate anaesthesia, combined with pneumatic methods (blowing and/or sucking) warranted further investigation.

Invertebrate anaesthesia through the use of controlled atmospheres is an established technique for the management of pests post-harvest (Kader & Ke, 1994; Suskiw, 2005). Carbon dioxide is often the material used, and has successfully been utilised for post-harvest control of aphids, thrips, beetles, flies, moths and mites, primarily in fruit, but also in some vegetable crops such as asparagus, broccoli and leafy vegetables (Mitcham *et al*., 2003). However, the aim of carbon dioxide in these instances has been to kill insects in the store rather than to immobilise them or dislodge them from produce. Carbon dioxide is approved as a commodity substance by the Pesticides Safety Directorate for this purpose in food storage practice. Consequently, the length of time needed to achieve control stretches into hours or days rather than seconds.

Use of carbon dioxide is a common technique for temporarily immobilising invertebrates by entomologists to ease handling of insects (Tatchell, 2005), and is used by SAC and other research organisations routinely. However, no attempts have been made to measure the concentrations of carbon dioxide needed to achieve anaesthesia using this technique; carbon dioxide is simply passed into a chamber where the insects are held and they very rapidly become immobile to allow them to be handled. Recovery is swift (within 10-30 seconds or so) and the insects show no side effects from their brief period of anaesthesia.

There have been several attempts to develop machines for the pneumatic control and removal of insects from crops (Lacasse *et al*., 2001; Vincent *et al*., 2003). Many of these have shown some promise for insect removal through the use of blowing and/or sucking on crops such as potatoes (Weintraub *et al*., 1996; Lacasse *et al*., 2001), celery (Weintraub *et al*., 1996) and strawberry (Vincent & Boiteau, 2001). It has been possible to demonstrate 100% removal of Colorado potato beetles from potato foliage using airstreams alone (Khelifi *et al*., 1995), although this level was not achieved under field conditions. These methods have been developed for insect control rather than simple removal or dislodging of insects from plants.

Lole (2002) developed a prototype ‘blower’ for use on baby-leaf salad crops. However, the agronomic features of the crop affected the efficacy of removal. Insect contaminants on spinach and red chard were removed at levels of up to 60%, whereas contaminant removal from Lollo Rosso and Oak-leaf lettuce proved more difficult (Lole, 2002).

The methods outlined above have not attempted to anaesthetise or relax the muscles of insects before attempting pneumatic removal or dislodging of invertebrates. Only limited research has been carried out to determine the mechanical forces need to dislodge insects from plants (Misener & Boiteau, 1993). Mechanical vibration of plants have been shown to

remove 90% of insects if the frequency and amplitude are optimised, and this may be exploiting the behavioural phenomenon known as thanatosis present in many insects (Boiteau & Misener, 1996).
Thanatosis is the behavioural reaction of insects where they simulate death and fall off plants when threatened, and studies of this behaviour are few and far between (Boiteau & Misener, 1996; Hozumi & Miyatake, 2005; Couturier *et al*., 2005). Insects exhibiting thanatosis become immobile and cease gripping the substrate that they are on, and often will curl themselves into a 'ball' shape. If they are on the slightest incline on a leaf, or if the leaf is disturbed, they will roll off the leaf and drop to the ground. Some insects may even actively run off a leaf to escape the perceived threat. Some insect contaminants of baby leaf salads (e.g. crickets) are known to exhibit thanatosis (Hozumi & Miyatake, 2005), and this behaviour may provide an opportunity for enhancing the removal of invertebrates from salads.

The overall commercial objective of this project is to provide the data necessary for the development of an engineering solution to the removal of contaminants from salad crops. Determining whether the major invertebrate contaminants exhibit thanatosis (playing 'dead') when disturbed, will provide insights into the methods that may be adopted to help dislodge them from plants.
Carbon dioxide is commonly used as an anaesthetic/relaxant in research on insects. Determining the concentrations of carbon dioxide needed to relax insects sufficiently for them to be dislodged from plants, coupled with measurements of the threshold forces required to dislodge insects from leaves (with and without carbon dioxide anaesthesia), will provide data for the application of fluid dynamics modeling to maximise the efficacy of airflow around plants to achieve the aim of dislodging contaminants.
Once the data outlined above has been obtained, research projects will be developed to identify where in the baby leaf salad harvesting process an engineering solution can be developed to minimise the presence of contaminants. The best method may be to dislodge the contaminants before cutting the crop, or perhaps once the crop is being graded. However, no decisions can be taken on which approach is best until there is a greater understanding of the behaviour of invertebrate contaminants to forces applied to them on the crop.

Materials and Methods

Source of invertebrates
Invertebrates were obtained from a variety of sources:

- Aphids (*Myzus persicae*) were obtained from SCRI and a culture maintained at SAC on swedes in the insectary.
- Ladybirds (*Coccinella septempunctata*) were obtained from CSL, York, and kept in containers in the insectary and fed on aphids.
- Large cabbage white butterfly caterpillars (*Pieris brassicae*) were obtained from Blades Biological Ltd. as eggs, and reared on swede plants.
- Slugs (the grey field slug, *Deroceras reticulatum*) were collected from various locations and kept at 5ºC and fed on slices of carrot.
- Ground beetles (*Pterostichus spp*.) were collected from the field from various locations in pitfall traps.

Source of leaves
The leaves used were chard leaves which were grown in the glasshouse from seed.

Measuring the force needed to dislodge invertebrates from leaves
The apparatus used for measuring the force necessary to dislodge an invertebrate from a leaf is shown in Fig. 1.
Five settings were used which relate to the height of the 'drop' of the arm, which held a plastic container that inside had a metal plate to which the leaf was attached via double-sided sticky tape (Fig. 2). The accelerative force (g) needed to dislodge an invertebrate was measured for 5 different settings using an accelerometer (Monitran 1010 MTN 10). The

output signal was conditioned as per manufacturer's specification and calibration sheet to provide a voltage output proportional to the g force experienced at the accelerometer. The voltage was captured and the peak detected using a portable storage scope. Note that all further force data presented are derived from the accelerometer outputs where the accelerometer is positioned in the rig, on the fixed member and not mounted on the leaf mount (due to physical size and weight of accelerometer likely to significantly modify any result if mounted in the leaf position). It is expected that there will be a level of attenuation at the position of the leaf/insect. A measurable estimate of this attenuation was carried out at one (lower) height setting using a miniature accelerometer (Entran) bonded by tape to the leaf mount. Using this set-up, the attenuation showed to be in the region between 1.5 and 2 for the specific setting. More consistent results were however obtained by bolting the Monitran accelerometer to the fixed member. In summary all force data presented herein relating directly to insect removal, are subject to an unspecified scaling error, although this does not prevent relative comparisons of all the dataset. Figure 3 shows an example of one of the height, and therefore g-force, settings used.

To maintain consistency of release, a switched electro-magnet was used to release the arm. This was operated by a button switch remote from the apparatus. Calibration measurements of g-force were performed and repeated to ensure consistency of results.

The overall force experienced by the invertebrate is the mass of the invertebrate in kg (based on average weight of the invertebrate) multiplied by 9.81, multiplied by the accelerative g force applied.

For example:-

- Invertebrate weight = 180mg
- Applied g force = 51g

The force experienced by the invertebrate is ((180 / 1000) / 1000)) x 9.81 x 51 , which is 0.090 Newtons, or 90 mN.

Fig. 1: Drop apparatus used to measure the force necessary to dislodge an invertebrate from a leaf.

Fig. 2: Close up of leaf in plastic container with plastic window

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Fig. 3: Example of one of the drop heights used to attempt to dislodge invertebrates from a leaf.

Twenty (20) individual invertebrates were tested at each of the 5 applied force settings. Caterpillars were split into small and large caterpillars, where small refer to 2nd instar, and large to 5th instar caterpillars. Slugs were separated into small and large individuals based on their body size: small were juveniles (< 10mm) and large were adults (> 10mm). Aphids were also split into two distinct groups: those which were not feeding on the leaf and those that were feeding and had their stylets inserted into the leaf surface.

When tests were carried out with carbon dioxide (CO_2), the gas was introduced into the plastic container via a gas syringe. Carbon dioxide was vented into a gas bag from a compressed CO_2 cylinder, and the CO_2 required was withdrawn from the gas bag using the gas syringe. An equivalent amount of air was withdrawn from the container using the same syringe. Concentration of CO_2 was varied by introducing specific amounts of the gas into the container (Fig. 4). Carbon dioxide concentrations were verified using a CO_2 meter (Gascard II, Edinburgh Instruments Ltd.). Replicate numbers were reduced to 12 for the CO_2 tests, and they were only carried out at two applied force settings, 3 and 4 (181g and 72g respectively). This was because settings 1 (480g) and 2 (331g) dislodged virtually all invertebrates, and setting 5 (51g) dislodged very few invertebrates.

Tests were carried out at room temperature (18ºC ± 2ºC) and 10ºC ± 2ºC. The tests at 10ºC were only carried out at applied force setting 3 (181g) for comparison with the same applied force setting at room temperature.

Assessing thanatosis

A moderate force setting (181g) was used to assess whether any invertebrates exhibited thanatosis. This was visually quantified as the invertebrate 'playing dead' and remaining immobile for several seconds after being subjected to mechanical stimulation. These tests were carried out on 10 individuals from each invertebrate group at room temperature 18ºC ± 2ºC and at 10ºC ± 2ºC.

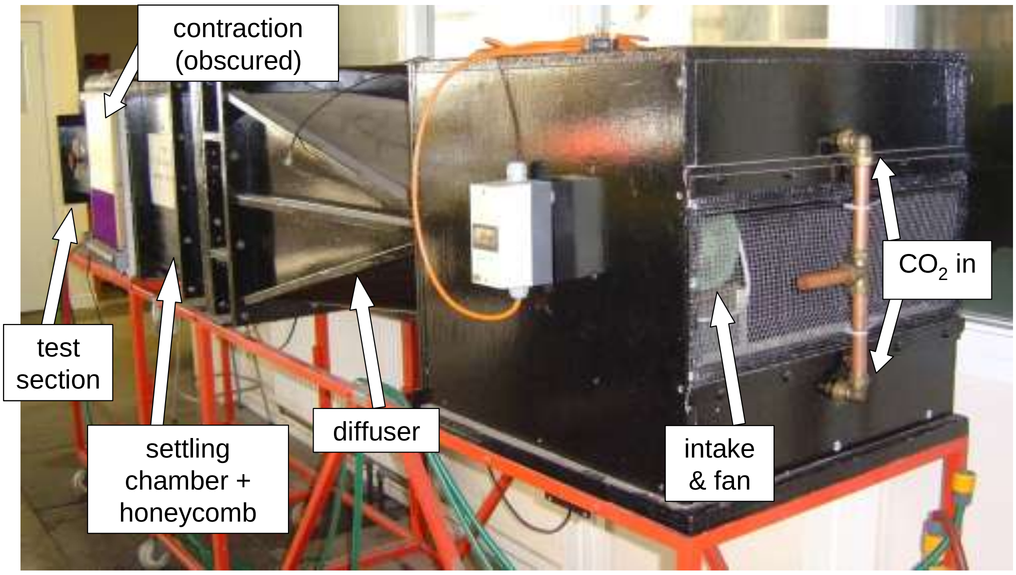
Fig. 4: Plastic container, leaf and invertebrate inside. Air volume removed and CO_2 introduced through the valves in the lid.

Statistical methods
Numbers of invertebrates dislodged at room temperature and 10ºC using were compared using Fisher's exact test based on the numbers dislodged at room temperature and 10ºC, and numbers not dislodged at these temperatures.
Numbers of invertebrates dislodged at room temperature (relative to total number) at the different applied force settings, with and without different CO_2 concentrations, and time exposed to the CO_2, were analysed as binomial proportions using logistic linear models. The logistic linear model included coefficients for force setting, carbon dioxide concentration and exposure time. (See Appendix for details of the models used).

Demonstration in a wind tunnel
This phase of testing was based around a small wind tunnel (Fig. 5). The tunnel was of conventional design, consisting of an intake chamber with axial fan, diffuser, settling chamber (with honeycomb flow-straightening), a contracting section, leading to the test section. Downstream of the test section, the tunnel vents to atmosphere. The test section has a square cross-section of 0.25 x 0.25m. The air speed was variable up to a maximum of c. 22 ms^{-1}, giving a maximum tunnel Reynolds number of c. 400000.

The flow speed was measured by hand-held anemometer. Carbon dioxide concentration was estimated by comparing volume flow rates at a fixed fan speed without, and then with the CO_2 valve open. Given that these tests were seeking indicative rather than definitive, quantitative conclusions, this method was considered satisfactory at this stage.

Fig. 5: Wind tunnel used to demonstrate effects of wind speed and CO_2 on invertebrates on leaves.



Carbon dioxide was introduced from a standard 65 litre vapour withdrawal cylinder of 99% industrial CO_2 via a 10 bar regulator and 22mm diameter pipes. The CO_2 was introduced immediately upstream of the fan (Fig. 5), ensuring strong mixing with air, in turn giving confidence in the uniformity of concentration at the test section.

The leaf on which the invertebrates were on was held in place on a small pedestal approximately at the centre line of the test section. Leaves were positioned to be approximately horizontal, with stalk at the "leading edge", and main leaf trailing downstream (Fig. 6).

Fig. 6: Leaf positioned on pedestal in the wind tunnel test section (flow from right to left).

The part of the test section (visible) in which the leaf was mounted is hinged and shown (above) in the partly-open position, which allowed access for positioning of the leaf and invertebrates.

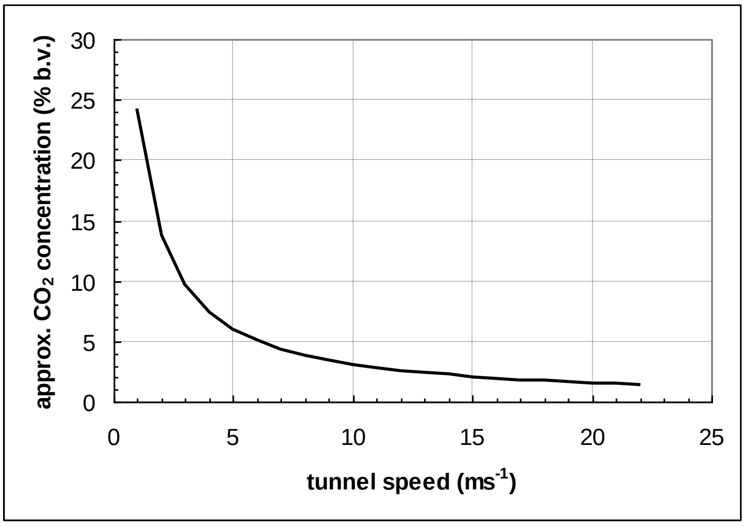
Two phases of tests were performed as follows:

Phase 1

Air-only tests were carried out with tunnel speed ramped up continuously to determine air-only tunnel speeds for invertebrate dislodgement. Tests were carried out with slugs, beetles and aphids.

Phase 2
These tests were carried with CO_2 introduced. In all tests, the CO_2 valve was opened fully, giving a flowrate of CO_2 of approximately 20 litres s^{-1}. Thus the concentration of CO_2 decreased with increasing tunnel speed according to Fig. 7.

Fig. 7: Variation of approximate CO_2 concentration with tunnel speed for Phase 2 tests.



Results and Discussion

Do insects exhibit thanatosis?
Ten invertebrates in each group were tested using a moderate applied force (Force setting 3 - 181g) and observed to see if they exhibited thanatosis. Thanatosis was measured as the invertebrate 'playing dead' (becoming immobile for several seconds) in response to mechanical stimulation.

Ladybirds
9 out if 10 ladybirds dropped off the leaf in response to stimulation, and they varied in their time for becoming mobile again – 10 to 60 seconds.

Caterpillars (large cabbage white butterfly)
The response depended on the size and location of the caterpillars on the leaf surface. Caterpillars near the edge of the leaf occasionally gripped the leaf edge.
Small caterpillars (second instar) – 6 out of 10 were dislodged from the leaf and showed signs of thanatosis, recovering after 10-30 seconds.
Large caterpillars (final instar) – 9 out of 10 caterpillars were dislodged from the leaf and showed signs of thanatosis, recovering after 10-30 seconds. The caterpillars would curl up into a ball on being stimulated and consequently lost their grip.
Some leaves where caterpillars had pupated were tested and the pupae and pre-pupae were not dislodged at all.

Aphids (peach-potato aphids)
The response varied depending on whether aphids were feeding or not. When feeding the aphids pierce the leaf surface with their stylets to reach phloem, so they are physically attached to the leaf.

Feeding aphids – 5 out of 10 aphids were dislodged from the leaf. Those that were dislodged showed evidence of thanatosis by being immobile after being dislodged for up to 30 seconds.
Non-feeding aphids – 8 out of 10 aphids were dislodged from the leaf and remained immobile for up to 30 seconds.

Ground beetles (Carabid beetles)
If beetles were near the edge of a leaf they could grip onto the leaf edge. All beetles not on the leaf edge demonstrated thanatosis for a few seconds.

Slugs (Grey field slug)
Two different sizes of slugs were studied: large (> 1cm) and small (< 1cm).
Only 5 out of 10 large slugs were dislodged. On disturbance, their bodies could be seen to contract and they appear to 'grip' the leaf more strongly than when they were relaxed.
The small slugs were more difficult to dislodge, with only 2 slugs being dislodged. Their bodies also looked to contract on disturbance.

All invertebrates tested exhibited some degree of thanatosis on being mechanically disturbed. Ladybirds, ground beetles and larger caterpillars were most likely to lose grip and 'play dead' when being disturbed. Aphids and slugs were less so, depending on whether aphids were feeding and the size of the slug.

Determining the force needed to remove invertebrates from leaves
A range of forces were applied to leaves which had invertebrates on them, to determine the minimum force necessary to dislodge the invertebrate. Whilst the accelerative force (g) was constant for each drop setting, the force experienced by each invertebrate (mN) is a combination of the accelerative force (g) and the weight of the insect.
Accelerative force (g) ranged from Setting 1 (480g) to 5 (51g) and 20 individuals of each invertebrate were tested at each force setting (Table 1). For caterpillars and slugs, there were separate tests for small and large individuals, and for aphids, distinction was made between aphids that were feeding on the leaf and those that were not.

In terms of accelerative force applied to the leaf, feeding aphids and small slugs required the greatest force (480g) to achieve the highest level of removal, and ground beetles the least (181g).
Using a logistic linear model of the binomial proportions of those dislodged and total number of invertebrates tested at each force setting, there is strong evidence ($P < 0.001$) that the number of each invertebrate species dislodged is influenced by the force setting. i.e. the greater the applied force the greater the probability of more invertebrates being dislodged.

Table 1: The percentage of invertebrates dislodged from a leaf when subjected to several accelerative forces using the drop apparatus

Percentage of invertebrates dislodged from the leaf					
Force setting	Ladybirds	Caterpillars	Aphids	Ground beetles	Slugs

(g)								
		Small	*Large*	*Not feeding*	*Feeding*		*Small*	*Large*
1 - 480g	100	100	100	100	90	100	95	100
2 - 331g	100	100	100	100	80	100	80	100
3 - 181g	90	70	85	95	55	100	30	45
4 - 72g	50	30	50	35	10	30	10	15
5 - 51g	10	15	15	20	5	5	5	5

The force experienced by the invertebrates is a function of the accelerative force applied and the weight of the invertebrate; the heavier the invertebrate, the greater the force experienced (Table 2).

The heavier the invertebrate, the greater the force experienced. Large caterpillars for example, experienced a force of 710 mN at applied force setting 3 (181g), whereas small caterpillars only experienced 9 mN at the same force setting (Table 2).

Table 2: The force (mN) experienced by the invertebrate taking into account accelerative force (g) and weight of the insect (mg)

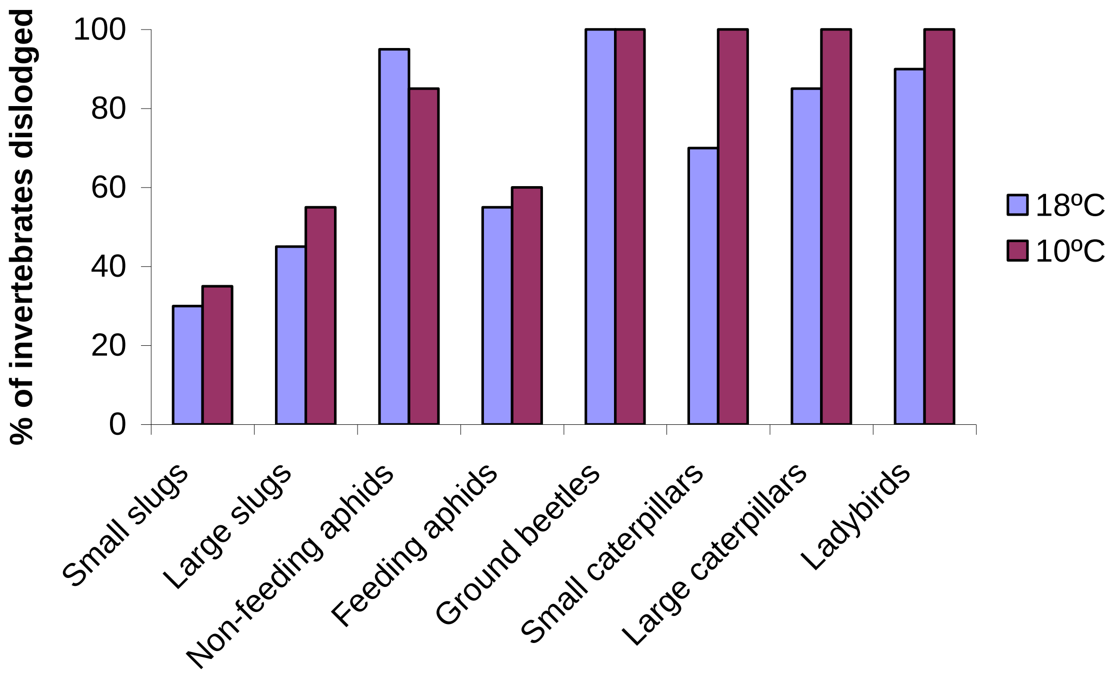
Force (mN) experienced by invertebrate (average weight in mg)								
Drop setting	*g*	*Ladybirds (30)*	*Caterpillars*		*Aphids (0.25)*	*Ground beetles (180)*	*Slugs*	
			Small (5)	*Large (400)*			*Small (10)*	*Large (80)*
1	480	141	24	1,884	1.2	848	47	377
2	331	97	16	1,299	0.8	584	32	260
3	181	53	9	710	0.4	320	18	142
4	72	21	4	283	0.2	127	7	57
5	51	15	3	200	0.1	90	5	40

Effect of temperature on the force needed to remove invertebrates

Lower temperatures (10ºC) tended to increase the numbers of invertebrates removed from leaves compared to 18ºC when the same accelerative force (181g) is applied to the leaf (Fig. 8).

For each invertebrate group (apart from non-feeding aphids and ground beetles) the proportion of invertebrates dislodged is significantly higher at 10ºC than at room temperature (18ºC) (Fisher's exact test, $P < 0.02$).

Fig. 8: The effect of temperature on removal of invertebrates from leaves receiving the same accelerative force (181g).



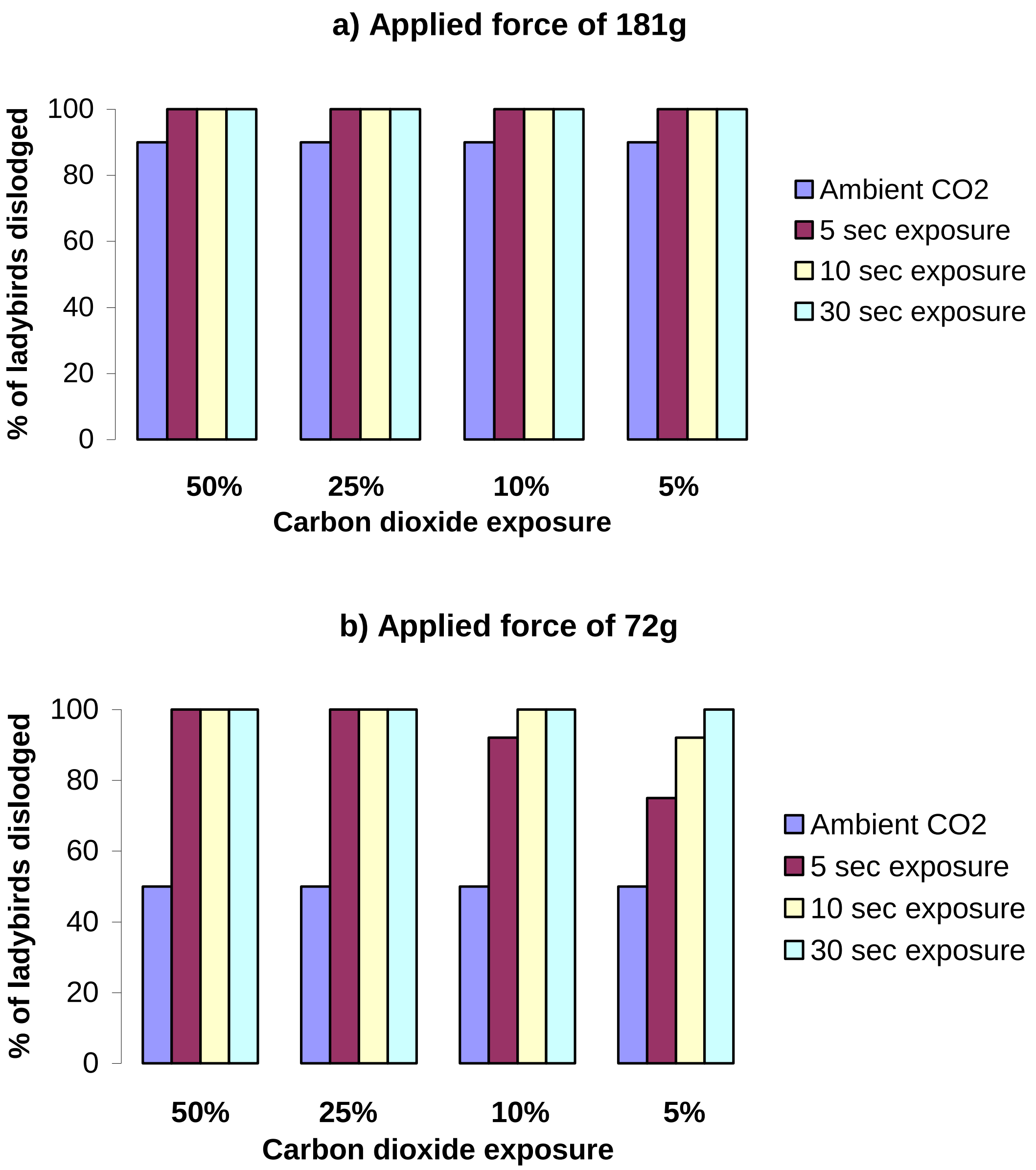
Effect of carbon dioxide concentration and duration of exposure on the force needed to remove invertebrates

Elevated carbon dioxide concentration and length of exposure significantly reduced the force required to remove invertebrates from leaves (Logistic linear model, $P < 0.001$).

Ladybirds

Ladybirds were significantly affected by exposure to elevated CO_2 concentrations ($P < 0.001$), and were removed from leaves at lower applied forces ($P < 0.005$) than at ambient CO_2 levels (Fig. 9). The duration of exposure to CO_2 had a significant impact; the longer the exposure to CO_2 the more likely the ladybirds were dislodged from the leaf ($P < 0.05$).

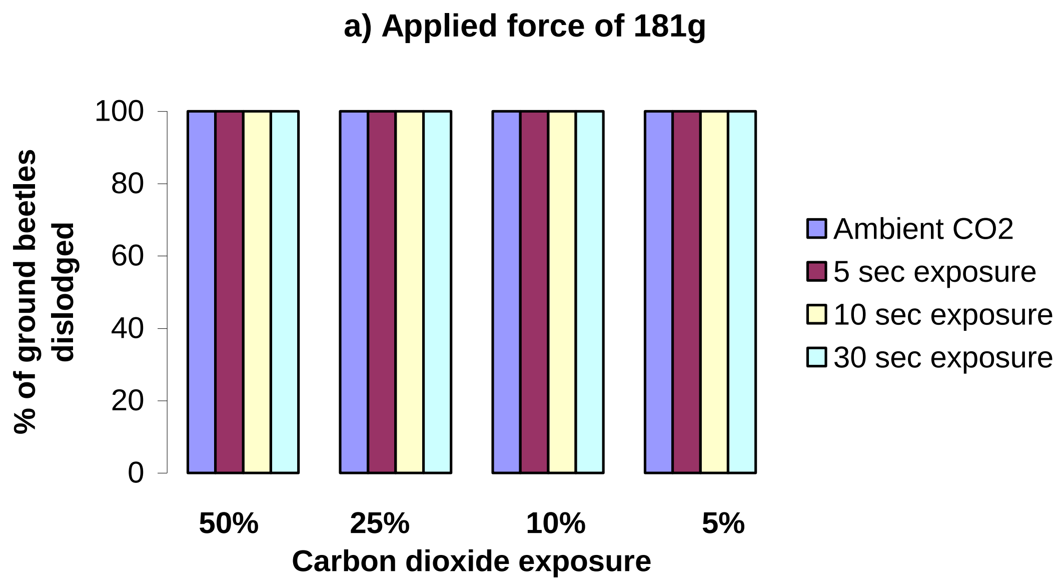
Fig. 9: Percentage of ladybirds dislodged from leaves at ambient, 50%, 25%, 10% and 5% CO_2 and exposure times of 5, 10, and 30 seconds. Applied force (g) of (a) 181g and (b) 72g.

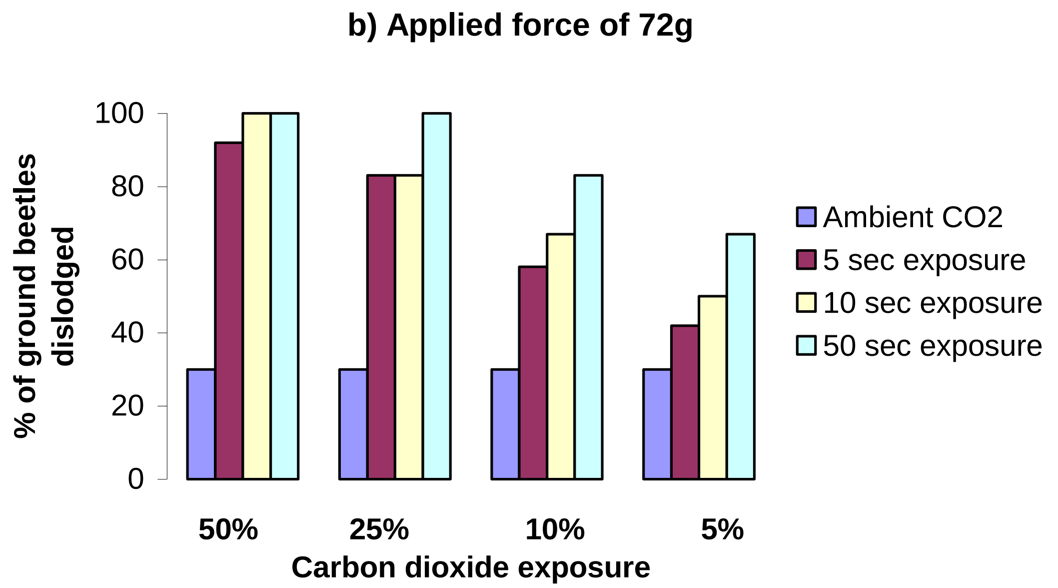


Ground beetles

Ground beetles were significantly affected by exposure to elevated CO_2 concentrations ($P < 0.001$), and were removed from leaves at lower applied forces ($P < 0.005$) than at ambient CO_2 levels (Fig. 10). The duration of exposure to CO_2 had a significant impact; the longer the exposure to CO_2 the more likely the ground beetles were dislodged from the leaf ($P < 0.05$).

Fig. 10: Percentage of ground beetles dislodged from leaves at ambient, 50%, 25%, 10% and 5% CO_2 and exposure times of 5, 10, and 30 seconds. Applied force (g) of (a) 181g and (b) 72g.

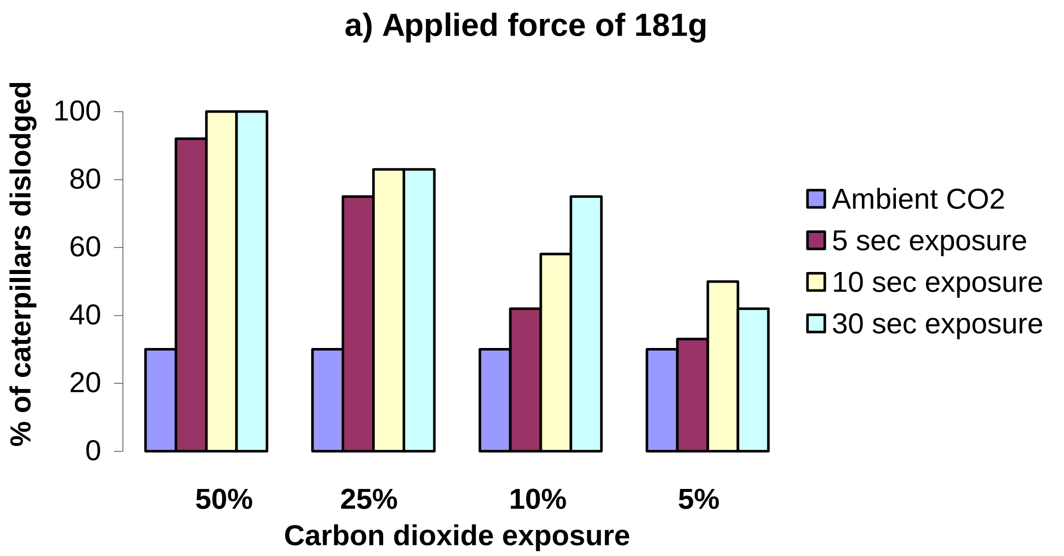




Caterpillars

Both small and large caterpillars were significantly affected by exposure to elevated CO_2 concentrations ($P < 0.001$), and were removed from leaves at lower applied forces ($P < 0.005$) than at ambient CO_2 levels (Figs. 11 and 12). The duration of exposure to CO_2 had a significant impact; the longer the exposure to CO_2 the more likely the caterpillars were dislodged from the leaf ($P < 0.05$).

Fig. 11: Percentage of small caterpillars dislodged from leaves at ambient, 50%, 25%, 10% and 5% CO_2 and exposure times of 5, 10, and 30 seconds. Applied force (g) of (a) 181g and (b) 72g.



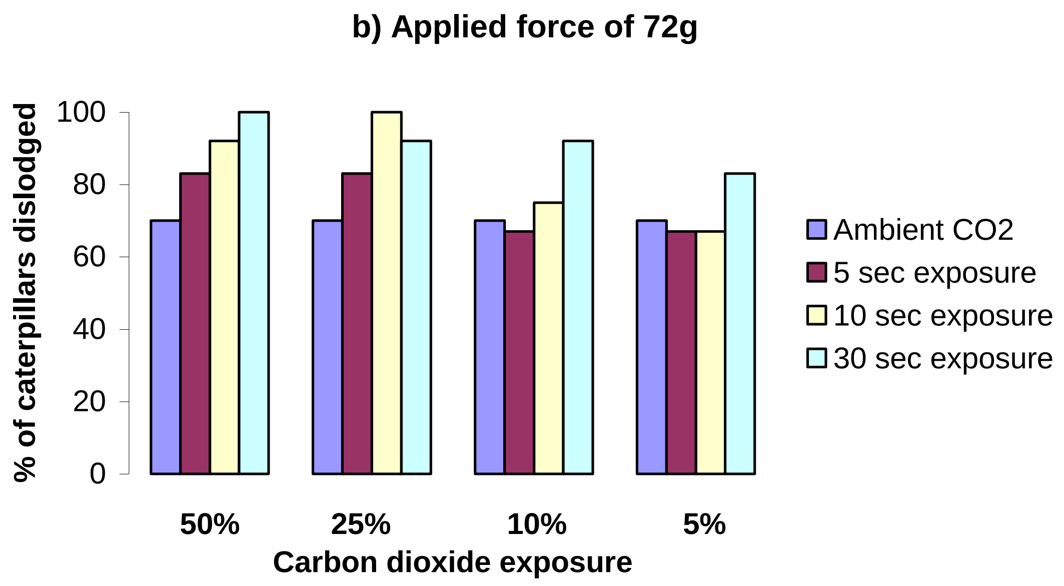
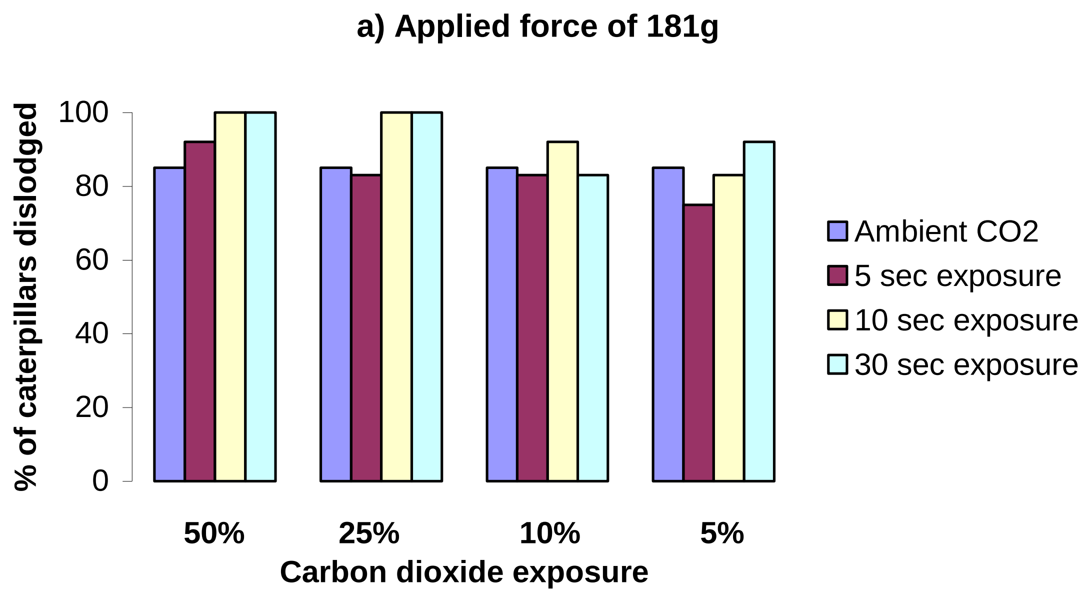
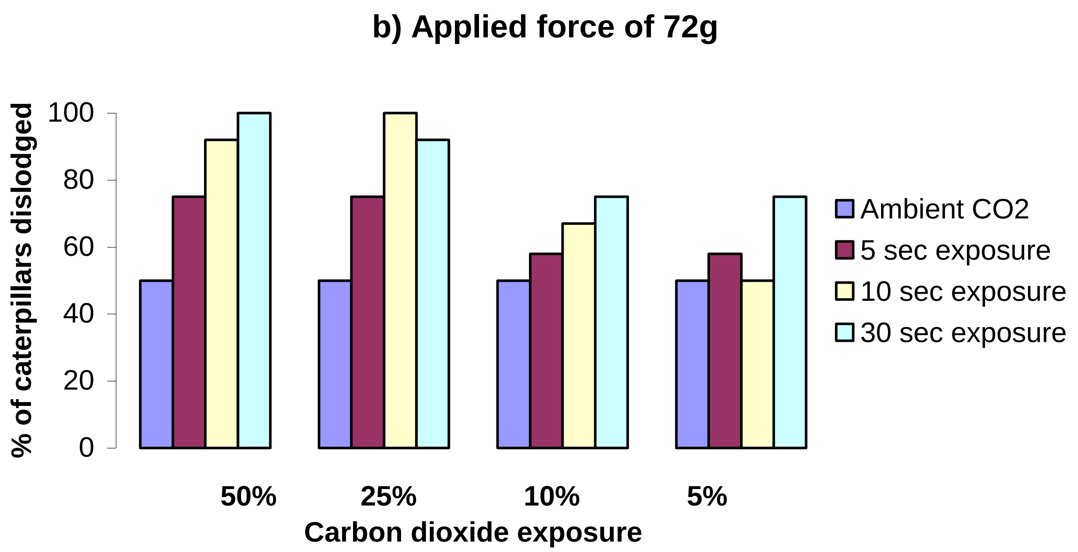


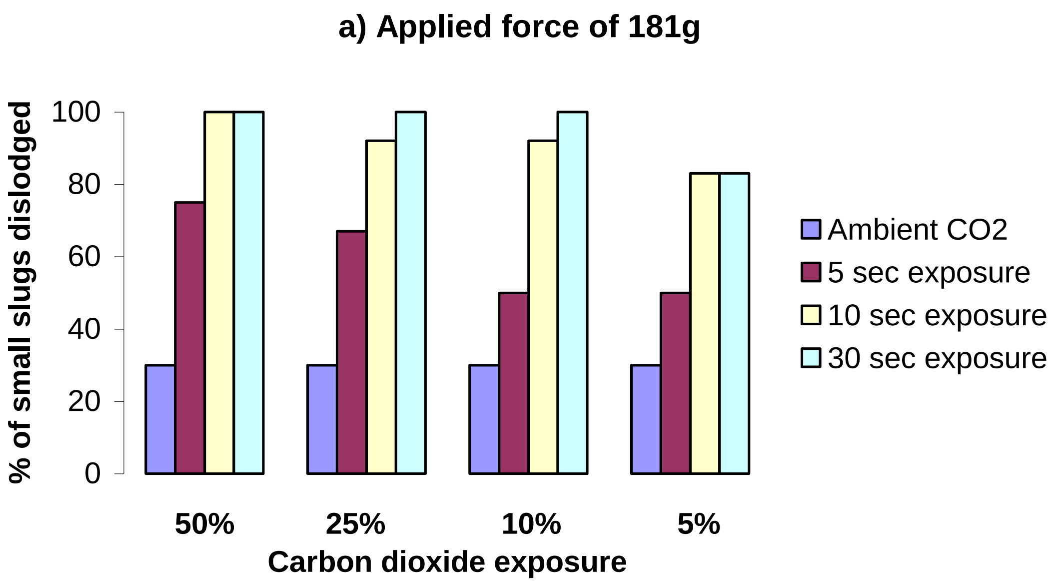
Fig. 12: Percentage of large caterpillars dislodged from leaves at ambient, 50%, 25%, 10% and 5% CO_2 and exposure times of 5, 10, and 30 seconds. Applied force (g) of (a) 181g and (b) 72g.


a) Applied force of 181g
% of caterpillars dislodged
100
80
60
40
20
0
50%
25%
10%
5%
Carbon dioxide exposure
Ambient CO2
5 sec exposure
10 sec exposure
30 sec exposure


b) Applied force of 72g
% of caterpillars dislodged
100
80
60
40
20
0
50%
25%
10%
5%
Carbon dioxide exposure
Ambient CO2
5 sec exposure
10 sec exposure
30 sec exposure

Slugs
Both small and large slugs were significantly affected by exposure to elevated CO_2 concentrations ($P < 0.001$), and were removed from leaves at lower applied forces ($P < 0.005$) than at ambient CO_2 levels (Figs. 13 and 14). The duration of exposure to CO_2 had a significant impact; the longer the exposure to CO_2 the more likely the slugs were dislodged from the leaf ($P < 0.05$).

Fig. 13: Percentage of small slugs dislodged from leaves at ambient, 50%, 25%, 10% and 5% CO_2 and exposure times of 5, 10, and 30 seconds. Applied force (g) of (a) 181g and (b) 72g.



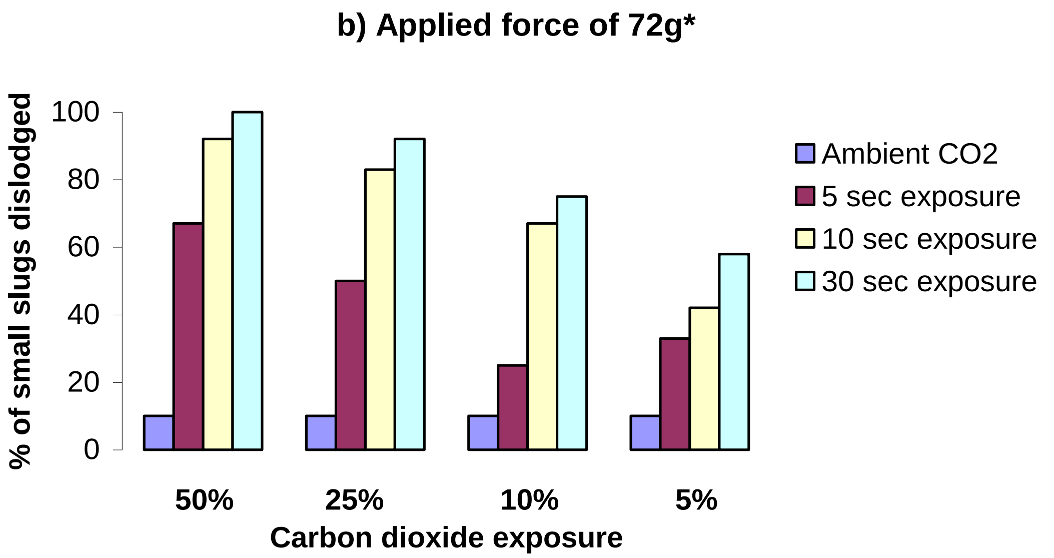
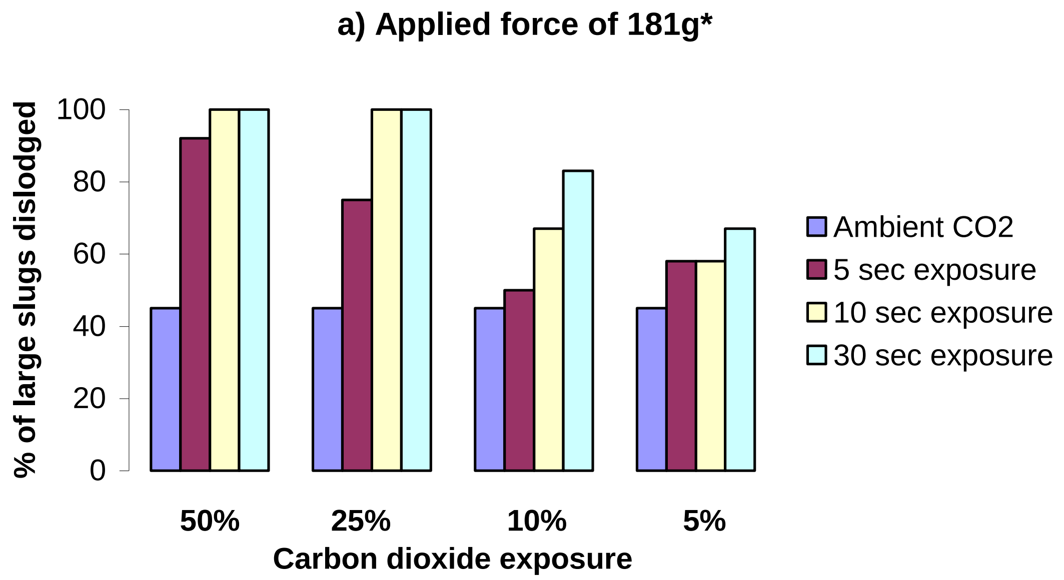
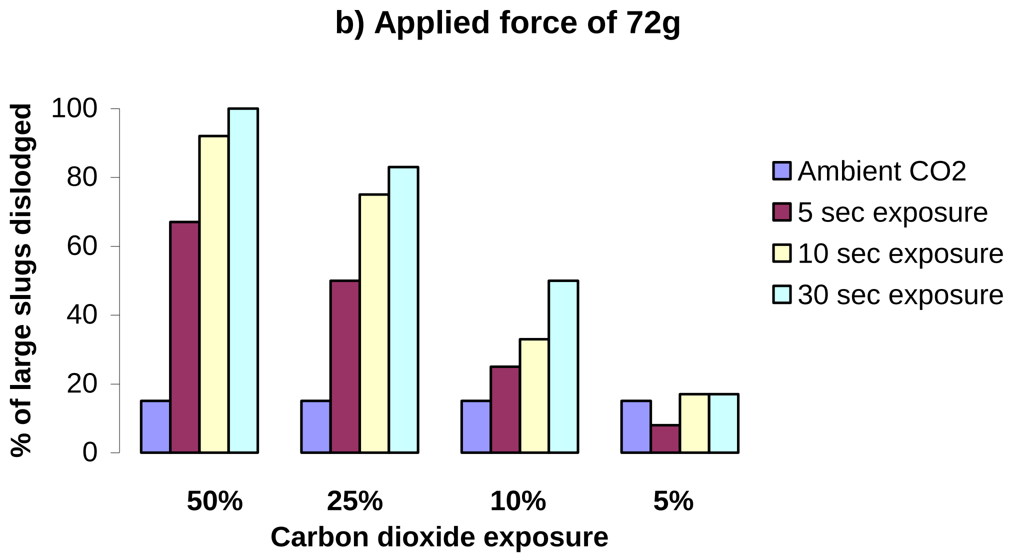


Fig. 14: Percentage of large slugs dislodged from leaves at ambient, 50%, 25%, 10% and 5% CO_2 and exposure times of 5, 10, and 30 seconds. Applied force (g) of (a) 181g and (b) 72g.





Aphids

Both non-feeding and feeding aphids were significantly affected by exposure to elevated CO_2 concentrations ($P < 0.001$), and were removed from leaves at lower applied forces ($P < 0.005$) than at ambient CO_2 levels (Figs. 15 and 16). The duration of exposure to CO_2 had a significant impact; the longer the exposure to CO_2 the more likely the aphids were dislodged from the leaf ($P < 0.05$).

Fig. 15: Percentage of non-feeding aphids dislodged from leaves at ambient, 50%, 25%, 10% and 5% CO_2 and exposure times of 5, 10, and 30 seconds. Applied force (g) of (a) 181g and (b) 72g.

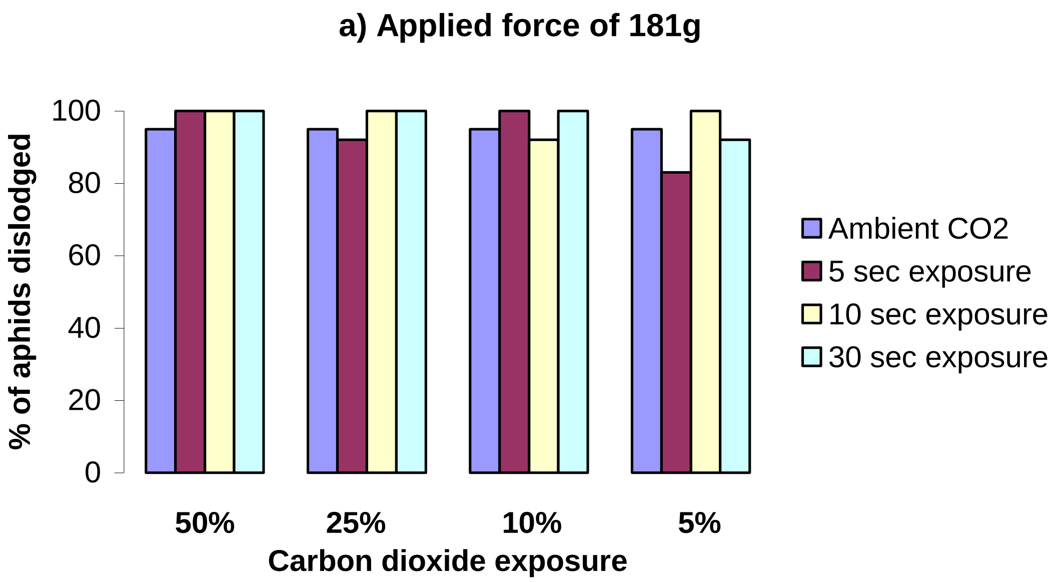
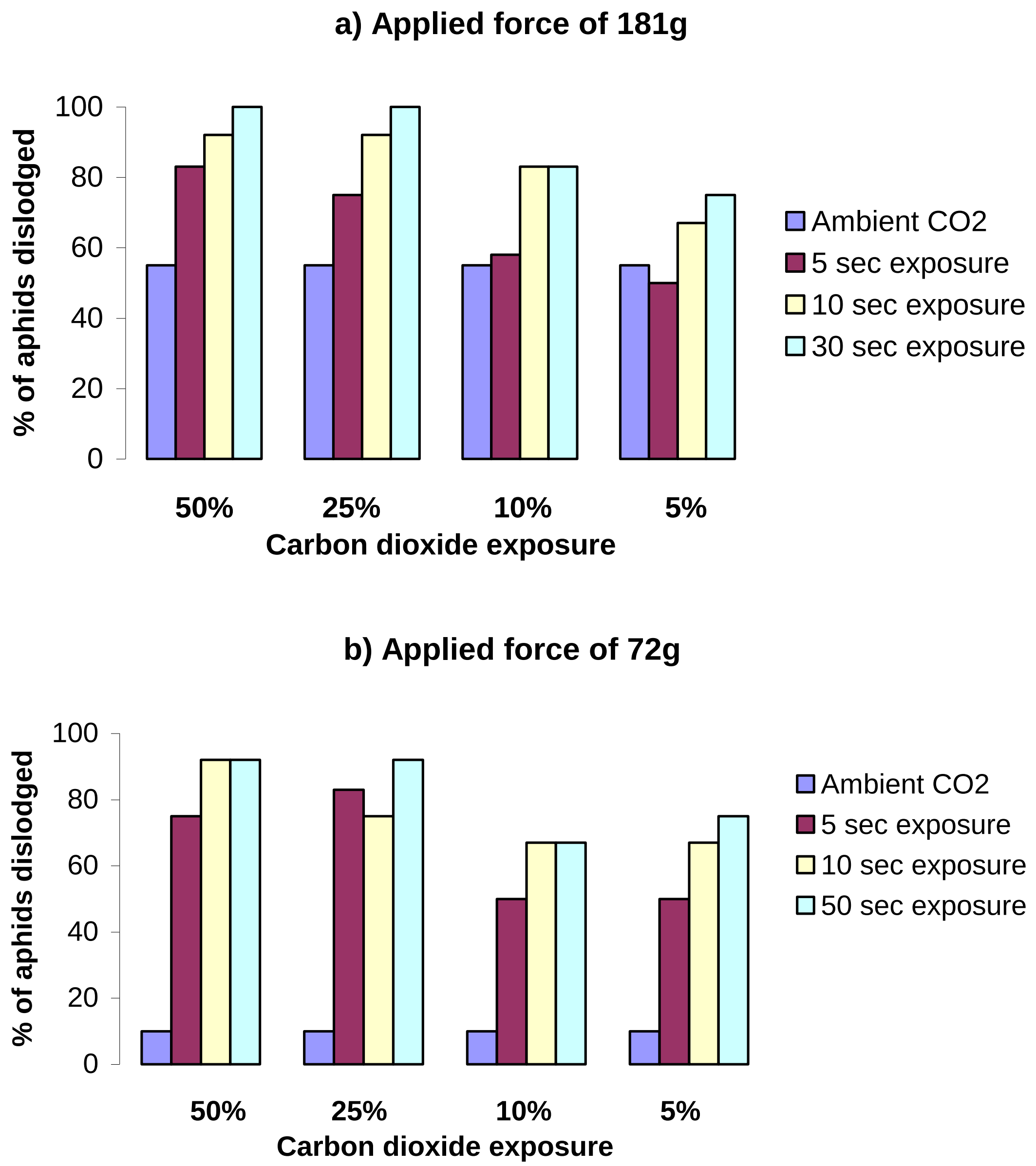


Fig. 16: Percentage of feeding aphids dislodged from leaves at ambient, 50%, 25%, 10% and 5% CO_2 and exposure times of 5, 10, and 30 seconds. Applied force (g) of (a) 181g and (b) 72g.



The results from the CO_2 tests are summarised in the table below. The minimum applied force at ambient CO_2 and elevated CO_2 level for each invertebrate is given, along with the optimal CO_2 concentration and length of exposure to CO_2 to dislodge 100% of invertebrates from a leaf.

For example, for large slugs, 100% were removed from leaves under ambient CO_2 levels with an applied force of 331g. At a CO_2 concentration of 25% and 10 seconds exposure, 100% of large slugs were removed with an applied force of 181g.

Table 3: Minimum Applied Force (g), CO_2 concentration (%) and duration of exposure (seconds) to achieve 100% removal of invertebrates from leaves

	Force (g) required to dislodge 100% of invertebrates from leaf			
Invertebrate	**At ambient CO_2**	**At elevated CO_2**	**Optimal CO_2 concentration* %**	**Duration of exposure to CO_2 (s)**
Small slugs	480	181	10	30
Large slugs	331	181	25	10
Non-feeding aphids	331	181	5	10
Feeding aphids	480	181	25	30
Ground beetles	181	72	25	30
Small caterpillars	331	181	25	10
Large caterpillars	331	72	25	10
Ladybirds	331	72	10	10

* Optimal CO_2 concentration refers to the concentration that led to 100% dislodging of invertebrates from the leaf.

Summary of elevated CO_2 tests (Table 3)
For each invertebrate group tested, exposure to elevated CO_2 levels led to a reduction in the applied force required to dislodge them from a leaf. For each invertebrate tested, the longer they were exposed to elevated CO_2 levels, the more likely they were to be dislodged by an applied force that did not dislodge them under ambient CO_2 levels. i.e. the increased exposure to elevated CO_2 led to anaesthesia and/or muscle relaxation in the invertebrate which facilitated their dislodging from leaves when mechanically stimulated.

Demonstration in a wind tunnel

Phase 1
The results of the Phase 1 tests are summarised below (Table 4). There was some variation in test conditions between nominally identical repeat tests due to variable level of invertebrate attachment to the leaf (e.g. whether or not aphid was feeding on the leaf; whether beetle was clinging to edge of leaf, or simply resting on flat part). Such observations are presented alongside the results. Note was also made of cases where excessive leaf-flapping was thought to have been the principal direct cause of dislodgement, rather than the direct drag from the air flow.

Table 4: Minimum wind tunnel speed (ms^{-1}) to dislodge slugs, beetles and aphids from a leaf:

Invertebrate	Tunnel speed for dislodgement (ms^{-1})	Observations
large slug	8.5	lots of leaf-flapping
large slug	8.5	smaller leaf; less flapping than in test above
large slug	10.5	no excessive leaf flapping
large slug	10.5	no excessive leaf flapping
beetle	10 – 11	beetle gripping edge of leaf
beetle	10.5	near edge
aphid	20+	"clever" hiding in lee in leaf surface
aphid	3	on middle of exposed, flat part of leaf
aphid	7.25	on middle of exposed, flat part of leaf
aphid	5	on middle of exposed, flat part of leaf
aphid	3	on middle of exposed, flat part of leaf
aphid	10	on middle of exposed, flat part of leaf

Phase 2

For the large slug, tests carried out according to the procedure of Phase 1 showed no discernable effect of 15s exposure to CO_2, with dislodgements recorded at approximately 10.5 ms^{-1} (at which tunnel speed the CO_2 concentration was only 3% b.v.).

Given the slug's ability to adhere passively to the leaf (possibly disguising any CO_2 effect), it was decided to concentrate on beetles. The experimental strategy adopted was to fix the tunnel speed at a relatively low value (3–3.5 ms^{-1}). At this speed, Phase 1 results would suggest little chance of a beetle being dislodged under air-only conditions. Twelve repeat tests were carried out, with the beetle subject exposed to 3–3.5 ms^{-1}, air-only, for 15s. In 12 separate tests with different beetles, only 3 beetles were dislodged (see Table 5).

Table 5: Beetle exposed to air-only and tunnel speed fixed at 3- 3.5 ms^{-1} for all tests

Invertebrate	Dislodged?	Observations / notes
beetle	yes	on flat part of leaf
beetle	yes	on flat part of leaf
beetle	no	on flat part of leaf
beetle	no	on flat part of leaf
beetle	no	on flat part of leaf
beetle	no	on flat part of leaf
beetle	no	clinging to edge of leaf
beetle	yes	on flat part of leaf
beetle	no	on flat part of leaf
beetle	no	on flat part of leaf
beetle	no	on flat part of leaf
beetle	no	on flat part of leaf

Below (Table 6) are the results of follow-up tests exactly as per the 12 air-only tests, but with c. 9% b.v. CO_2 now introduced for an exposure time of 15 seconds.

Table 6: Beetle exposed to air + CO_2 (9% b.v.) for 15s. Tunnel speed fixed at 3- 3.5 ms^{-1} for all tests

Invertebrate	Dislodged?	Observations / notes
beetle	no, but...	... fell off shortly after air flow was stopped
beetle	yes, after 10s	
beetle	yes, after 7–8s	
beetle	yes, after 5s	
beetle	yes, after 10s	
beetle	yes, after 10s	
beetle	yes, after 5s	
beetle	yes, after 7s	
beetle	yes, after 8–10s	

Four further air-only tests were performed to “close the loop” and ensure that earlier observations were broadly repeatable (Table 7).

Table 7: Repeatability tests – beetle exposed to air only again. Tunnel speed fixed at 3- 3.5 ms^{-1} for all tests:

Invertebrate	Dislodged?
beetle	no
beetle	no
beetle	no
beetle	no

The air-only results of Phase 1 were broadly in line with expectations from the drop tests. Without detailed knowledge of the drag characteristics of the pests, precise comparisons cannot be made. Nevertheless, estimates using the standard formula for form drag

$$F_D = \tfrac{1}{2} c_D \, \rho A u^2$$

with c_D = 1 gave dislodgement tunnel speeds of 10 – 20 ms^{-1} for all invertebrates tested.

The drop test data with CO_2 is not directly comparable to the wind tunnel tests due to the very much higher CO_2 concentrations used in the drop tests (up to 50% in the drop tests compared to 3 – 10% in wind tunnel).

Taken together, Phase 1 and Phase 2 tests showed evidence of the desired effect of exposure to CO_2 on beetles.

Summary of wind tunnel tests

In tests carried out in a wind tunnel, tunnel speeds of 10-20 ms^{-1} were required to dislodge beetles and aphids and at ambient CO_2 levels. Beetles exposed to air + 9% CO_2 for 15 seconds were dislodged at tunnel speeds of 3 - 3.5 ms^{-1} whereas beetles exposed to air only at tunnel speeds of 3 - 3.5 $ms\text{-}^{1}$ were not dislodged.

The tests have provided sufficient evidence of desired effect of CO_2 and are sufficiently in line with the drop test data to suggest that a more extensive and detailed study is merited. Such a study would investigate the required time of exposure for given CO_2 concentrations and involve a more extensive set of multiple-repeated tests.

Conclusions

- Several common invertebrate contaminants of vegetables exhibit thanatosis ('play dead') when disturbed. This does appear to depend where the invertebrate is on the leaf. If at the leaf edge for example, beetles, ladybirds and caterpillars tended to grip the leaf edge and were less likely to be dislodged when the leaf was mechanically disturbed, whereas if they were in the middle of the leaf, they had nothing to grip onto and immediately 'played dead' and fell off the leaf.
- The applied accelerative force to the leaf necessary to dislodge invertebrates was determined for each invertebrate group. This ranged from an applied force of 480g to 181g depending on the invertebrate.
- The larger the invertebrate, the less applied force was necessary. This is a function of the total force experienced by the invertebrate, which is a product of the applied force and the weight of the insect - the heavier the insect, the greater the force experienced by the insect. So, for example, small slugs required a greater applied force than larger slugs to dislodge them from leaves.
- Invertebrates tend to be dislodged easier at lower temperatures.
- Exposing invertebrates to elevated CO_2 levels for time periods of 5, 10 or 30 seconds led to an increase in their dislodging compared to ambient CO_2 levels. For all invertebrates, the applied force needed to dislodge 100% of them from leaves was lower at elevated CO_2 levels than at ambient CO_2.
- A research proposal will be developed after consultation and feedback from growers and industry with a view to devise a prototype system based on CO_2 to remove contaminants from salads.

Technology transfer

No technology transfer activities have taken part during the project, however, the results from this project will be presented at a meeting of the Outdoor Salads and Radish R&D group (British Leafy Salads Association technical group) in October 2007. An article for HDC News will also be prepared.

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Appendices

Results of applied force tests

Table A1: The effect of different accelerative forces on the removal of invertebrates from salad leaves

No. of invertebrates removed from the leaf (out of 20)								
Force setting (g)	**Ladybirds**	**Caterpillars**		**Aphids**		**Ground beetles**	**Slugs**	
		Small	*Large*	*Not feeding*	*Feeding*		*Small*	*Large*
1 - 480g	20	20	20	20	18	20	19	20
2 - 331g	20	20	20	20	16	20	16	20
3 - 181g	18	14	17	19	11	20	6	9
4 - 72g	10	6	10	7	2	6	2	3
5 - 51g	2	3	3	4	1	1	1	1

Table A2: The percentage of invertebrates dislodged from leaves at applied force of 181g at room temperature (18ºC) and 10ºC

% of invertebrates removed from the leaf		
Invertebrate	*18ºC*	*10°C*
Small slugs	30	35
Large slugs	45	55
Non-feeding aphids	95	85
Feeding aphids	55	60
Ground beetles	100	100
Small caterpillars	70	100
Large caterpillars	85	100
Ladybirds	90	100

% of ladybirds removed from the leaf at different CO_2 concentrations and exposure time (seconds)													
Force setting (g)	Ambient CO_2	50% CO_2			25% CO_2			10% CO_2			5% CO_2		
		5	10	30	5	10	30	5	10	30	5	10	30
181	90	100	100	100	100	100	100	100	100	100	100	100	100
72	50	100	100	100	100	100	100	92	100	100	75	92	100

% of small caterpillars removed from the leaf at different CO_2 concentrations and exposure time (seconds)													
Force setting (g)	Ambient CO_2	50% CO_2			25% CO_2			10% CO_2			5% CO_2		
		5	10	30	5	10	30	5	10	30	5	10	30
181	70	83	92	100	83	100	92	67	75	92	67	67	83
72	30	92	100	100	75	83	83	42	58	75	33	50	42

% of large caterpillars removed from the leaf at different CO_2 concentrations and exposure time (seconds)													
Force setting (g)	Ambient CO_2	50% CO_2			25% CO_2			10% CO_2			5% CO_2		
		5	10	30	5	10	30	5	10	30	5	10	30
181	85	92	100	100	83	100	100	83	92	83	75	83	92
72	50	75	92	100	75	100	92	58	67	75	58	50	75

% of non-feeding aphids removed from the leaf at different CO_2 concentrations and exposure time (seconds)													
Force setting (g)	Ambient CO_2	50% CO_2			25% CO_2			10% CO_2			5% CO_2		
		5	10	30	5	10	30	5	10	30	5	10	30
181	95	100	100	100	92	100	100	100	92	100	83	100	92
72	35	92	100	100	75	75	100	58	83	92	42	58	67

% of feeding aphids removed from the leaf at different CO_2 concentrations and exposure time (seconds)													
Force setting (g)	Ambient CO_2	50% CO_2			25% CO_2			10% CO_2			5% CO_2		
		5	10	30	5	10	30	5	10	30	5	10	30
181	55	83	92	100	75	92	100	58	83	83	50	67	75
72	10	75	92	92	83	75	92	50	67	67	50	67	75

% of ground beetles removed from the leaf at different CO_2 concentrations and exposure time (seconds)													
Force setting (g)	Ambient CO_2	50% CO_2			25% CO_2			10% CO_2			5% CO_2		
		5	10	30	5	10	30	5	10	30	5	10	30
181	100	100	100	100	100	100	100	100	100	100	100	100	100
72	30	92	100	100	83	83	100	58	67	83	42	50	67

% of small slugs removed from the leaf at different CO_2 concentrations and exposure time (seconds)

Force setting (g)	Ambient CO_2	50% CO_2			25% CO_2			10% CO_2			5% CO_2		
		5	10	30	5	10	30	5	10	30	5	10	30
181	30	75	100	100	67	92	100	50	92	100	50	83	83
72	10	67	92	100	50	83	92	25	67	75	33	42	58
51	5	25	50	67	17	17	58	0	25	58	8	8	50

% of large slugs removed from the leaf at different CO_2 concentrations and exposure time (seconds)													
Force setting (g)	Ambient CO_2	50% CO_2			25% CO_2			10% CO_2			5% CO_2		
		5	10	30	5	10	30	5	10	30	5	10	30
181	45	92	100	100	75	100	100	50	67	83	58	58	67
72	15	67	92	100	50	75	83	25	33	50	8	17	17

Example of statistical methods

Data analysed using Genstat for Windows.

Force setting and temperature - small caterpillars
Number dislodged at ambient temperature (relative to total number) to be analysed as binomial proportions using logistic linear models
MODEL [DISTRIBUTION=binomial; LINK=logit; DISPERSION=1] Number_dislodged; NBINOMIAL=Total_number

Logistic linear model for dependence on force setting
FIT [PRINT=model,summary,estimates; FPROB=yes; TPROB=yes] Force_setting

*** Summary of analysis ***

	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
Regression	1	62.027	62.027	62.03	<.001
Residual	3	3.986	1.329		
Total	4	66.014	16.503		

Dispersion parameter is fixed at 1.00

There is strong evidence (P < 0.001) that the number dislodged is influenced by the force setting. The residual deviance is similar to the residual degrees of freedom, so the fit of the model appears good.

*** Estimates of parameters ***

estimate s.e. t(*) t pr.

Constant	6.47	1.25	5.18	<.001
Force_setting	-1.763	0.337	-5.23	<.001

Compare numbers dislodged for force setting 3 at ambient temperature and 10°C using Fisher's exact test:
based on numbers dislodged at ambient and low temperatures and numbers not dislodged at these temperatures.
VARIATE [VALUES = 14,20,6,0] Tablefreqs
FEXACT2X2 Tablefreqs

***** Fisher's Exact Test *****

One-tailed significance level 0.010
Mid-P value 0.005

Two-tailed significance level
Two times one-tailed significance level 0.020
Mid-P value 0.010
Sum of all outcomes with Prob<=Observed 0.020
Mid-P value 0.010

The significance probabilities in a one-sided and a two-sided test are approximately 0.01 and 0.02, so there is evidence that the expected proportion dislodged is higher at 10°C than at the ambient temperature.

Force setting, CO_2 concentration and time of exposure
Number dislodged relative to total number to be analysed as binomial proportions using logistic linear models.
MODEL [DISTRIBUTION=binomial; LINK=logit; DISPERSION=1] Number_dislodged; NBINOMIAL=Total_number

Logistic linear model includes coefficients for force setting, carbon dioxide concentration and exposure time
FIT [PRINT=model,summary,estimates; FPROB=yes; TPROB=yes] Force_setting + CO2 + Exposure

*** Summary of analysis ***

	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
Regression	3	51.17	17.0567	17.06	<.001
Residual	20	14.91	0.7456		
Total	23	66.08	2.8731		

Dispersion parameter is fixed at 1.00.

* MESSAGE: The following units have large standardized residuals:

Unit	Response	Residual
1	10.00	-2.01

There is strong evidence (P < 0.001) that the number dislodged is influenced by the three variables. The residual deviance is small (less than the residual degrees of freedom), so the fit of the model appears good.

*** Estimates of parameters ***

	estimate	s.e.	t(*)	t pr.
Constant	2.77	1.13	2.45	0.014
Force_setting	-0.913	0.310	-2.94	0.003
CO2	0.0639	0.0127	5.04	<.001
Exposure	0.0360	0.0150	2.40	0.016

The significance probabilities for the individual coefficients suggest that force setting, carbon dioxide concentration and exposure time all affect the probability that a caterpillar is dislodged.

To check model assumed, plot residuals from fit against carbon dioxide concentration and exposure time:
nonlinearity in either plot suggests that model assumed is not appropriate"

```
RKEEP [RMETHOD=deviance] RESIDUALS = dresidual
XAXIS WINDOW = 1; TITLE = 'CO2 concentration'
YAXIS WINDOW = 1; TITLE = 'Deviance residual'
DGRAPH [TITLE='Residuals from logistic regression on force setting, CO2 and\
exposure plotted against CO2 concentration'] dresidual; CO2
XAXIS WINDOW = 1; TITLE = 'Exposure time'
DGRAPH [TITLE='Residuals from logistic regression on force setting, CO2 and\
exposure plotted against exposure'] dresidual; Exposure
```

The plots suggest some curvature in the relationship between the logit of the probability that a caterpillar is dislodged and carbon dioxide concentration and exposure time. The choices of the values used in the experiment also suggest that a non-linear relationship is expected. These variables are replaced in the model by their (natural) logarithms, and the model is re-fitted.

Fit alternative logistic linear model using (natural) logarithms of carbon dioxide concentration and exposure time

```
  CALCULATE log_CO2 = LOG(CO2)
  CALCULATE log_Exposure = LOG(Exposure)
  FIT [PRINT=model,summary,estimates; FPROB=yes; TPROB=yes]\
  Force_setting + log_CO2 + log_Exposure
```

*** Summary of analysis ***

		mean	deviance	approx	
	d.f.	deviance	deviance	ratio	chi pr
Regression	3	52.79	17.5971	17.60	<.001
Residual	20	13.29	0.6645		
Total	23	66.08	2.8731		

Under the alternative model, there is slightly stronger evidence that the number dislodged is influenced by the three variables. The residual deviance is smaller than before.

*** Estimates of parameters ***

	estimate	s.e.	t(*)	t pr.
Constant	0.27	1.27	0.21	0.833
Force_setting	-0.933	0.315	-2.96	0.003
log_CO2	1.118	0.199	5.60	<.001
log_Exposure	0.576	0.218	2.64	0.008

The significance probabilities for the individual coefficients again suggest that all three variables affect the probability that a caterpillar is dislodged.

```
Check fit of alternative model
RKEEP [RMETHOD=deviance] RESIDUALS = dresidual
XAXIS WINDOW = 1; TITLE = 'CO2 concentration'
DGRAPH [TITLE='Residuals from logistic regression on force setting, log CO2 and\
log exposure plotted against CO2 concentration'] dresidual; CO2
XAXIS WINDOW = 1; TITLE = 'Exposure time'
DGRAPH [TITLE='Residuals from logistic regression on force setting, log CO2 and\
log exposure plotted against exposure'] dresidual; Exposure
```

The plots for the alternative model show that the curvature has been reduced by regressing on the logarithms of carbon dioxide concentration and exposure time: this appears to be a better model.