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Non-destructive measurement of dormant bud respiration rates

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Abstract

The lack of an indicator of the state of bud development during the dormant period has been a major difficulty in studying the effects of winter chilling on subsequent shoot growth and flowering. We considered that respiration rate (R_D) might provide such an index, so developed a technique for the non-destructive measurement of the R_D of individual dormant buds of kiwifruit (*Actinidia deliciosa*). A closed configuration gas exchange system was used. The low R_D of dormant buds required the use of an unusually small system volume. As a consequence, it was necessary to modify the conventional closed system so that most of the system volume could be sampled for analysis. Increases in CO_2 concentration during a measurement were determined by injecting gas samples into a stream of air flowing through an infrared gas analyser. The technique was found to be reliable even at R_D as low as 20 pmol s^{-1}. Error analysis showed that under normal operating conditions the coefficient of variation was approximately 3 %. With two operators, measurements could be made at the rate of one bud every four minutes. The ability to make non-destructive measurements has the advantage of enabling us to make sequential measurements on individual buds and monitor subsequent development. The system could be readily adapted to other woody fruit species, providing that gas-tight seals can be established on individual buds. Use of the system is illustrated by measuring the temperature response of the R_D of dormant buds grown under contrasting conditions. All buds showed increasing R_D with increasing temperature in the range 15 to 25 °C. Buds which had grown in the coolest region, where bud break is usually the earliest, had the highest R_D when measured during early spring.

Additional key words: *Actinidia deliciosa*; CO_2 concentration; temperature.

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Introduction

During dormancy many developmental changes occur. A major difficulty in studying the processes involved is that there are no external clues to changes in the state of the bud during the period when environmental influences are having a large impact. In species such as kiwifruit (*Actinidia deliciosa*), structural changes associated with flower formation cannot be detected by microscopic examination until about three weeks before bud break (Linsley-Noakes and Allan 1987, Snowball and Walton 1992) although the timing of responses to contrasting environments show that changes must occur earlier than this. Cole *et al.* (1982) found differences in R_D of dormant buds of two pear cultivars which differed in their time of flowering. The midwinter R_D of the early-blooming species, *Pyrus calleryana*, was double that of *P. communis*, the late blooming species. The bud respiration rates were measured using a destructive technique and the tissue of several buds contributed to each measurement. This study prompted us to investigate whether changes in the respiration rate of dormant kiwifruit buds could be used as an index of bud state considerably earlier than the microscopically visible changes just before bud break.

Other studies have also found links between respiration rate and dormancy but these have included considerably more than bud tissue. Using destructive measurements of entire shoots, Young (1990) found that the respiration rate of apple shoots responded more strongly to temperature as chilling increased. The R_D of shoot tips of Douglas Fir seedlings varied during bud development (Fielder and Owens 1992). In an entire Hinoki forest tree studied over a three year period Paembonan *et al.* (1991) found that the daily respiration rate was lowest in the winter during the dormant period and that the sensitivity of night-time R_D to temperature was inversely related to air temperature. We found one study where there was no close association between rest and rate of respiration: Hatch and Walker (1969) measured the uptake of oxygen by peach and apricot buds which had been cut in half.

We considered that the monitoring of respiration to provide an index of bud state in kiwifruit would be most useful if the measurements could be made non-destructively and on individual buds. The advantage of being able to follow changes in individual dormant buds and to relate these changes to their later development was seen to be considerable. We considered a range of techniques to determine the R_D of buds by measuring the rate of release of CO_2 using infra-red gas analysis.

We elected to use a closed configuration gas exchange system rather than an open system (see Larcher 1969, Jarvis *et al.* 1971, Long and Hallgren 1985 for comparison of system designs). The flux of CO_2 produced by individual dormant buds was expected to be much lower than is normally experienced in gas exchange measurements of plant material because both the rate of CO_2 production and the amount of tissue involved are small. An open system would require the control and measurement of extremely low rates of flow to attain a sufficiently high CO_2 differential to achieve reliable measurements. Low flow rates would introduce long measurement delays because of the time required to flush the relatively large volume measurement chambers of commercially available infrared gas analysers.

A closed system where the analyser is included in a recycling loop has the

advantage of not requiring the manual transfer of gas samples (McPherson *et al.* 1983, Sheoran and Boyer 1989) but the total volume would then be too large to attain the necessary measurement accuracy in an acceptable time. Instead, it was necessary to inject air samples into a stream of air of known and stable CO_2 concentration which was continually flowing through the analyser. This approach has been used in a range of applications (Larson and Kershaw 1975, Atkins and Pate 1977, Clegg *et al.* 1978). In all cases only a small proportion of the system volume was extracted.

Materials and methods

Principle of operation: In a closed system, the change in concentration of the gas being measured is directly proportional to the flux of gas introduced to the system or removed from it by the tissue being measured, and inversely proportional to the volume of the system.

The respiration rate of a bud, R_D [mol s^{-1}] is given by:

$$R_D = \frac{\Delta C \, N\nu \, T_0 \, P}{\Delta t \, V_M \, T \, P_0} \qquad (1)$$

where ΔC = the rise in CO_2 concentration [mol mol^{-1}] from the beginning of the measurement (C_1) to the end (C_2), Δt = the duration of the measurement [s] from the start time (t_1) to the finish time (t_2), $N\nu$ = system volume [cm^3], V_M = the mol volume, 22.414 cm^3 mol^{-1} at standard temperature and pressure, T_0 = standard temperature, 273.15 K, T = ambient temperature [K], P_0 = standard pressure, 101.3 kPa, P = ambient pressure [kPa].

To achieve the high sensitivity required for measuring R_D a low system volume was needed. A sample injection system was used to avoid the need for the measurement cuvette to form part of the system volume: It was necessary for the sample to be as large a proportion of the system volume as possible without allowing ambient air to enter the system while it was being extracted. A futher design requirement was that the bud aperture was exposed to normal CO_2 concentrations while the system was being sealed around the aperture and that the seal could be tested prior to measurement. To meet these requirements we designed a system perhaps best described as having a dynamic closed configuration because the set volume of air involved in the measurement flows past the bud during the measurement. The calculation of R_D from measurements made with this system can still be made using Eq. 1.

Dynamic closed system: The simplest form of a closed system involves a container of known volume ($N\nu$) sealed over the tissue being measured (Fig. 1*A,B*). The difference between the concentration (C_1) at the start of the measurement (t_1) and the concentration (C_2) at the end of the measurement (t_2) is used to calculate the rate of gas exchange. In our dynamic closed system, the volume of gas involved in the measurement ($N\nu$) was allowed to flow through the bud chamber. At the start of the experiment this air, which had an initial CO_2 concentration of C_1, started flowing

through the bud chamber (Fig. 1*C*). Part way through the measurement, some of this volume (B) contained CO_2 molecules released by the respiring bud and consequently had an increased CO_2 concentration (Fig. 1*D*). The remainder of the volume (A) was still at its initial concentration (C_1). By the end of the measurement (Fig. 1*E*), all of the volume of air involved in the measurement ($N\nu$) had passed the bud and was raised to the final concentration (C_2). A sample of this air was then introduced to the gas analyser for the measurement of C_2. The initial CO_2 concentration (C_1) was determined prior to the start of the experiment. As this air was supplied from a compressed air cylinder its CO_2 concentration was stable and it required only infrequent checking.

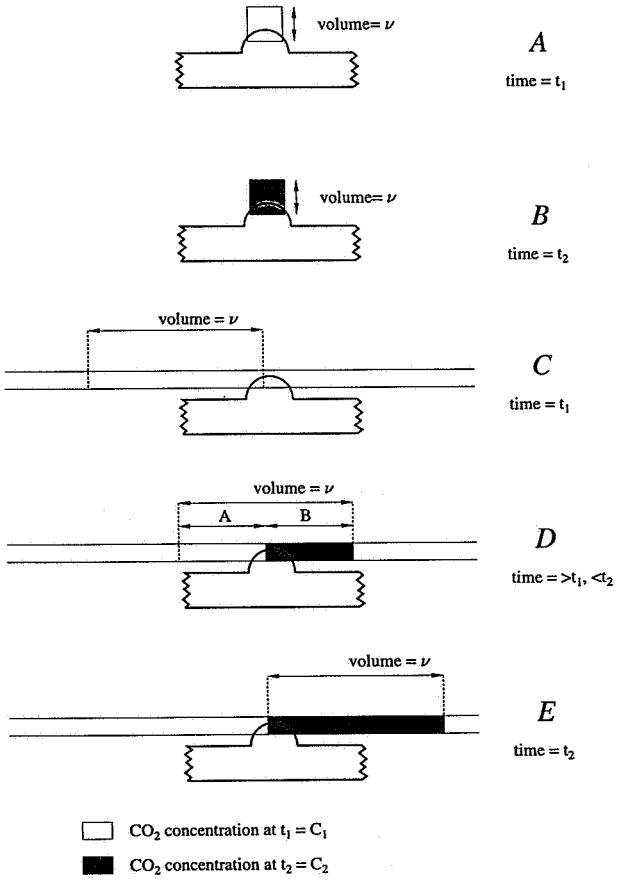

Fig. 1. Representation of 'closed' measurement systems attached to a single dormant bud. The simplest form is shown: (*A*) at the start of a measurement; and (*B*) at the end of a measurement. The 'dynamic closed' system is shown: (*C*) at the start of a measurement (*D*) part way through a measurement and (*E*) at the end of a measurement. In each case: the start of a measurement is at time t_1 when the CO_2 concentration is C_1; and the end of a measurement is at time t_2 when the CO_2 concentration is C_2.

System description: The system consisted of: a small bud chamber with an orifice which could be sealed against the bud aperture of an individual bud (Fig. 2*A*), a syringe pump for flushing air of known CO_2 concentration through the chamber at a constant rate and collecting it in a gas tight syringe (Fig. 2*A*), an infrared gas analyser being continuously flushed by an airstream of known concentration and into which the contents of the syringe could be injected (Fig. 2*B*), and a digital multimeter which measured and stored the peak reading from the infrared gas analyser (Fig. 2*B*).

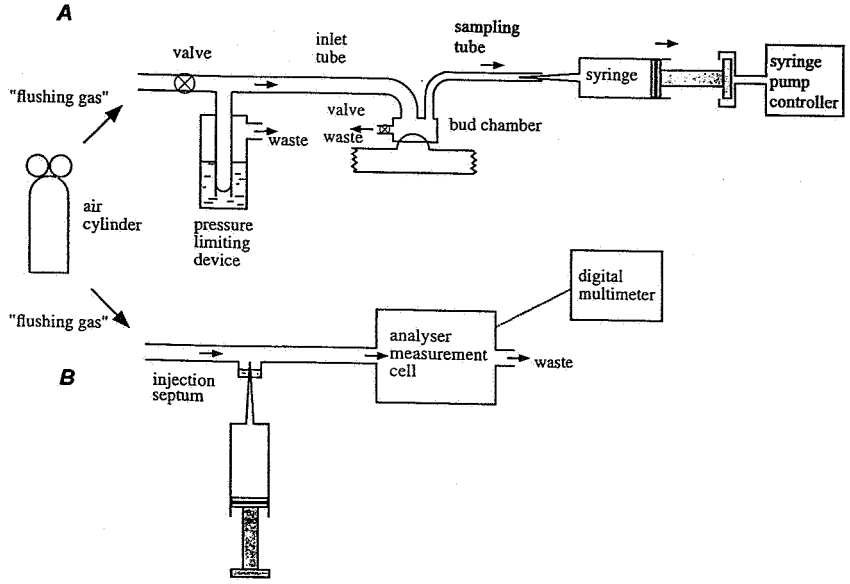

Fig. 2. Schematic diagram of the 'dynamic closed' air system (not to scale) showing: (*A*) the air supply to the bud chamber flowing through the pressure limiting device, the bud chamber attached to a dormant bud, and the syringe pump which extracts the gas sample for analysis; (*B*) the gas-tight syringe injecting the air sample into the airstream which flows through the infrared gas analyser, the digital multimeter for measuring the peak signal which results from the injection of the air sample.

Bud chamber: The bud chamber was designed to be of minimal volume so that most of the system's volume was within the syringe which held the sample. The brass chamber had a hole (5 mm i.d.) in the base, which was sealed around the periphery of the bud aperture (Fig. 3*A*) using a ring of sealant (*Blu-Tack, Bostik Australia Pty.*, Thomastown, Victoria, Australia) (Fig. 3*B*).

A polytetrafluoroethylene (PTFE) tube (3 mm i.d.) supplied air to the chamber and an exhaust port allowed the air to flow to waste from the chamber during the flushing phase which preceded each measurement. The chamber was flushed at a flow rate that ensured the CO_2 concentration was elevated by no more that 5 % of the concentration change that occurred during the subsequent measurement. This prevented significant differences developing between the actual initial CO_2 concentration and the assumed value (C_1). This required flow rates of about 0.5 to 5 $cm^3\ s^{-1}$. The maximum pressure rise in the inlet tube was set at 10 mm liquid paraffin (approximately 9 mm water gauge) to minimise the likelihood of leaks through the

Blu-Tack seal.

Each measurement was initiated by closing the exhaust which depressed a microswitch initiating the measurement period in synchrony with the end of the flushing period. During the measurement period, air was extracted at a constant and pre-determined rate through a small diameter PTFE tube (0.5 mm i.d.) which was sealed with a push-fit onto the tapered needle of a gas-tight syringe. Air flowed into the chamber through the inlet port, which remained open at all times, to replace the air being extracted. However, the flow rate (approximately 0.05 $cm^3 s^{-1}$) was much smaller than during the flushing phase. The excess inlet air, which was being supplied at a constant rate from a gas cylinder, ran to waste through a pressure-head relief system.

Air was extracted by the gas-tight syringe until a pre-set volume was reached. The syringe was then manually detached from the small diameter PTFE tube and injected through a septum into a stream of gas flowing through an infrared gas analyser. The peak response of the analyser was then recorded. The peak response was used in preference to the integral of the change in signal because it was easy to instrument using the maximum/minimum feature of the digital multimeter, and a measurement could be completed without waiting for the entire gas sample to pass through the measuring chamber of the analyser.

Chamber clamp: A clamp (Fig. 3*B*) was designed which could attach to canes of between 6 and 17 mm diameter. It provided a stable attachment on which bud chamber could be mounted. A screw adjustment on the chamber mount allowed the chamber to be tensioned against the *Blu-Tack* seal which surrounded the bud aperture. A second adjustment allowed the face of the chamber to be tilted so that it was in the same plane as the face of the bud cover which surrounded the bud aperture (Fig. 3*B*). The design of the chamber clamp was critical because it had to be stable enough to allow the chamber to be held securely in place without risk of leaks developing in the seal, yet able to be quickly attached and removed each time a measurement was made.

Pressure-limiting device: The glass pressure-limiting device controlled the pressure of the flushing gas by allowing excess gas to bubble to waste through a 10 mm head of liquid paraffin when the bud chamber exhaust port was closed. This limited thepressure-head to approximately 9 mm water gauge at the chamber. To test for leaks in the bud chamber seal prior to making a measurement, the outlets from the bud chamber and the inlet to the pressure-limiting device were closed briefly. Any rise of the paraffin meniscus in the bubbler indicated leaks. A change in volume of less than 0.01 cm^3 (< 0.2 % of the total system volume, v) could be detected easily.

Syringe pump: During each measurement, air was drawn at a constant rate into a gas-tight syringe which had a glass barrel with a PTFE end on the plunger (model *1005RN*, 5.0 cm^3, *Hamilton Company*, Reno, Nevada, U.S.A.). This was achieved by mounting the syringe in a cradle and using a stepping linear actuator (starting force 125 N, step 0.025 mm, maximum travel 170 mm) to extract the syringe plunger for a set distance, at a pre-determined rate. An electronic controller activated the stepping linear actuator. The 5 cm^3 of gas were extracted over a period which ensured that the

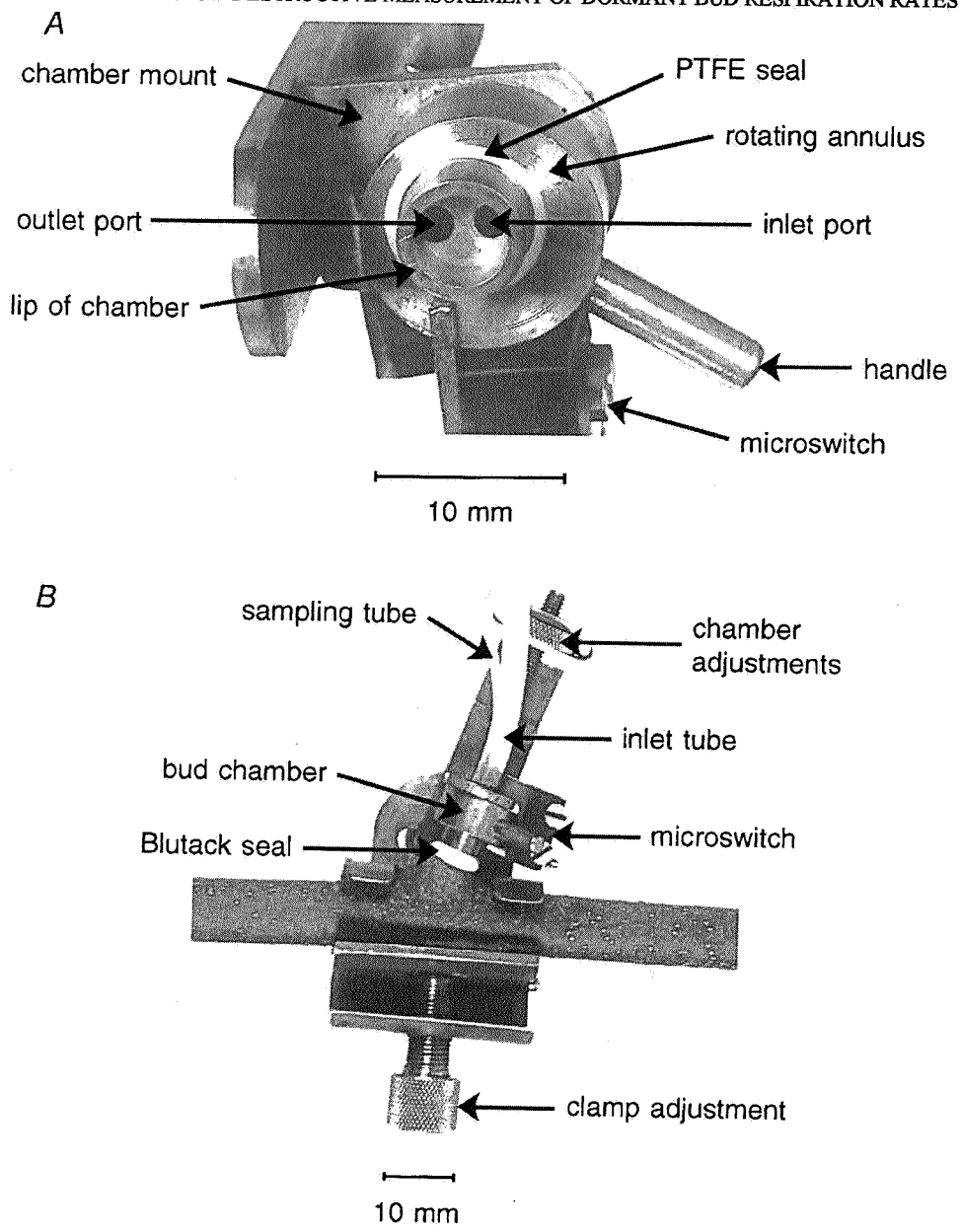

Fig. 3. The bud chamber and clamp. (*A*) An oblique view showing: the chamber orifice and the PTFE seal which separates the body of the chamber from the rotary valve which controls the exhaust port. Note the eccentric outer surface of the rotary valve that is used to activate the microswitch. The switch synchronises the closing of the exhaust port and the start of the measurement phase. (*B*) Side elevation showing the chamber clamped onto a dormant bud. The lower knurled knob is used to clamp firmly on the cane. The upper knurled knob is used to tension the chamber against the *Blu-Tack* seal. A third knob (unseen, behind the chamber) is used to adjust the angle of the chamber to the face of the bud. The larger diameter PTFE tube supplies the flushing gas, the smaller attaches to the syringe and extracts the gas sample. The rotary valve is shown in the open position (exhaust port is unseen at the back of the chamber).

CO_2 concentration rise in the system remained between 20 and 100 mmol mol^{-1}. The lower limit was determined by a compromise between the measurement accuracy required and the time taken for a measurement. The upper limit of CO_2 rise was set well below the concentration that might cause feedback inhibition of respiration. The length of the measurement period was set prior to each measurement based on the expected respiration rate of the bud.

Gas analysis: Each sample of gas was injected into a stream of air (the 'flushing gas') flowing continuously, at a set rate, through the measurement cell of an infra-red gas analyser (model *LI-6252, LICOR*, Lincoln, Nebraska, U.S.A.). The transient peak in the analogue signal from the analyser was measured using a multimeter which had a maximum/minimum function (Dual Display Multimeter, model *Fluke 45, Philips NZ*, Auckland, New Zealand). The peak response of the analyser was proportional to the difference in the concentration between the injected sample and the flushing gas. The flushing gas was supplied from a compressed air cylinder which had a concentration of CO_2 close to ambient and was also used for flushing the bud chamber.

The infrared gas analyser was operated in 'absolute' mode with the reference cell sealed. The zero and sensitivity were set so that the full scale range was from approximately 290 to 400 cm^3 m^{-3}. A further calibration was required to determine the sensitivity of the analyser to samples of injected gas. This sensitivity depended not only on the absolute calibration of the analyser, but also on: the signal averaging time for the gas analyser and the multimeter maximum/minimum function; and the volume of the sample injected. Because the volume of the sample was smaller than the volume of the measurement cuvette, the analyser response to injection was in direct proportion to the volume injected.

Measurement sequence: With flushing gas flowing through the bud chamber at the appropriate rate, the chamber was sealed over the bud aperture.A syringe was filled with flushing gas, installed in the syringe pump, and connected to the fine PTFE tube used to sample air from the bud chamber.

Once the chamber seal had been tested, the syringe pump pushed the flushing gas through the fine PTFE tube and into the chamber. This ensured that the gas in the chamber, tube, needle and any deadspace in the syringe was at a known CO_2 concentration. The syringe pump then extracted a gas sample stopping automatically when the syringe was full. This sample was then injected into the analyser immediately after the maximum/minimum function on the digital multimeter was activated. The maximum reading (determined by the CO_2 concentration of the sample) and minimum readings (determined by the CO_2 concentration of the carrier gas) were then used to calculate the respiration rate according to Eq. 1. With two operators, measurements could be made at a rate of one bud every four minutes.

Measurements were made in low ambient irradiances (*ca.* 10 μmol m^{-2} s^{-1}) and the bud was further shaded by the cane clamp. Tests comparing measurements at 10 μ mol m^{-2} s^{-1} with those made in darkness, showed not detectable differences between them.Consequently we have used the abbreviation R_D throughout.

Results and discusion

System tests

Zero checks: When the system was tested while sealed on to an inert surface in place of a bud, a small positive apparent R_D was often recorded. This system error was repeatable and was higher with higher syringe filling rates. During each experiment these 'zero errors' were recorded and the indicated respiration rate corrected. At R_D of 20 pmol s^{-1}, which was towards the lower end of the range for dormant buds, the corrections were approximately 15 % of the reading. As the R_D increased to 100 pmol s^{-1} and above, the correction fell to 3 % or less of the reading.

The dependence of the zero error on the syringe filling rate showed that CO_2 was entering the system at a rate related to the pressure drop in the syringe. The error could be made to increase if the system was exposed to the high ambient CO_2 concentrations. Since the error tended to increase with increased use of each syringe, we concluded that the error was due to leakage between the plunger and the barrel of the, so called, gas-tight syringes.

Injection volume: The response of the analyser was directly proportional to the volume of sample injected in the range from 1 to 10 cm^3 with a carrier gas flow rate of 3.33 cm^3 s^{-1}. The volume of the measurement cell of the analyser was 11.9 cm^3. We standardised on an injection volume of 5 cm^3. A larger volume would have given a proportionally larger analyser response but, for a given length of measurement on the bud, a proportionally smaller CO_2 differential.

Gas flow rate: The flow rate of the analyser flushing gas was set at 3.33 cm^3 s^{-1}. This was fast enough for the analyser to respond quickly to the injection of a sample, and low enough to conserve the cylinder of compressed air that was used to provide a steady flow of gas of known and stable CO_2 concentration.

Signal averaging time: The signal averaging time of the *LICOR* infra-red gas analyser was under software control. Longer sampling times reduced the short-term noise in the signal, but at the same time reduced the height of the peak response to an injected sample (data not presented). The standard deviation of measurements made by repeatedly injecting samples of the same concentration showed no detectable net effect of the averaging times in the range 1 to 10 s. We standardised on a setting of 1 s. The multimeter was used on its default measurement rate of 5 samples per second.

System specifications

Volume: The volume of the system was calculated from linear measurements of each of the components with the exception of the syringe (Table 1). The syringe contributed the largest single volume to the system. This was measured by filling it with water and measuring the mass change. The initial volume of the system at t_1 is that of the bud chamber, the PTFE sampling tube and the syringe needle (a total of 0.16 cm^3). The increase in the system volume during a measurement (between t_1 and t_2) was the volume of the syringe swept by the plunger (4.98 cm^3). The volume (v)

that is appropriate for use in calculating R_D is the total system volume (5.15 cm^3).

Table 1. The volume of each of the system components.

Item	Volume [cm^3]	Comment
Bud chamber	0.06	Calculated from linear measurements
Sampling tube and needle	0.10	Calculated from nominal dimensions
Syringe	4.99	From mass gain upon filling with water
Total final volume	5.15	Used in calculation of R_D

Range and speed of measurement: Values of R_D ranged from 10 pmol s^{-1} to over 200 pmol s^{-1}. The lower limit of measurement was determined by the required accuracy and the time that could be spent on each measurement. With our total system volume of 5.15 cm^3 the measurement of 18 pmol s^{-1} would take 247 s for a rise in CO_2 concentration of 20 μmol mol^{-1} (Table 2). We preferred to obtain a concentration rise of at least this level to have an acceptable analyser signal:noise ratio. It was possible, however, to make useful measurements at lower differentials, particularly when comparing populations of buds which had markedly different R_D. The measurement of 1720 pmol s^{-1} would take 12.3 s for a rise in CO_2 concentration of 100 μmol mol^{-1}. Our system was not configured to permit measurements to be made over smaller intervals than this. When buds were at the stage of development when external changes were just visible, the R_D often exceeded this value. In such cases the CO_2 concentration rise exceeded 100 μmol mol^{-1}.

Error analysis

Repeability: The uncertainty in each of the variables used to calculated Eq. 1 can be described using their variance and then used to estimate the uncertainty in the R_D rate ($\sigma^2_{R_D}$):

$$\sigma^2_{R_D} = \left(\frac{\partial R_D}{\partial \Delta C}\right)^2 \sigma^2_{\Delta C} + \left(\frac{\partial R_D}{\partial \Delta t}\right)^2 \sigma^2_{\Delta t} + \left(\frac{\partial R_D}{\partial v}\right)^2 \sigma^2_{v} + \left(\frac{\partial R_D}{\partial V_M}\right)^2 \sigma^2_{V_M} \qquad (2)$$

The variance of the CO_2 differential was determined from repeat measurements made during routine calibration of the analyser sensitivity to injections of known concentrations of calibrations gas. Each calibration curve was based on 5 different concentrations with repeat measurements at each concentration ($\sigma^2_{\Delta C} = 9\times10^{-14}$ mol^2 mol^{-2}). The variance of the volume of the system was determined from a series of 10 measurements of the volume of the syringe as described earlier ($\sigma^2_v = 5.04\ 10^{-5}$ cm^6). The variance of the measurement interval was calculated assuming a normal distribution and consequently that the standard deviation was 0.68 of the range. The range was calculated assuming an error of + 0.5 counts in the operation of the stepping linear actuator used to extract the plunger of the sample syringe ($\sigma^2_{\Delta t} = 1.81\ 10^{-5}$ s^2). The variance of the molar volume was calculated similarly, assuming a

deviation from the nominal temperature of up to ±1 K, and assuming a deviation from the nominal barometric pressure of ± 0.25 kPa ($\sigma^2_{V_M} = 1.4 \times 10^4$ cm^6 mol^{-2}).

Table 2. A sample from the table used to determine the appropriate duration of measurement (Δt) for a given expected bud respiration rate (R_D). Values are given for a CO_2 concentration rise of 20 and 100 mmol mol^{-1} which were the preferred lower and upper limits (system volume = 5.15 cm^3).

R_D [pmol s^{-1}] at ΔC = 20 μmol^{-1}	100 μmol mol^{-1}	Δt [s]
343	1720	12.3
115	573	37.0
34	173	123.5
18	86	247.0

The partial derivatives shown in Eq. 2 were determined from Eq. 1 and used to calculate the contribution of each variable to the variance of R_D.

The coefficient of variation of R_D was calculated for a range of measurement intervals and R_D (Fig. 4). Under the normal operating conditions defined in Table 2, the coefficient of variation would have been a maximum of approximately 3 %. When R_D values were low, the duration of the experiment was incresed to maitain this high repeatability of measurements.

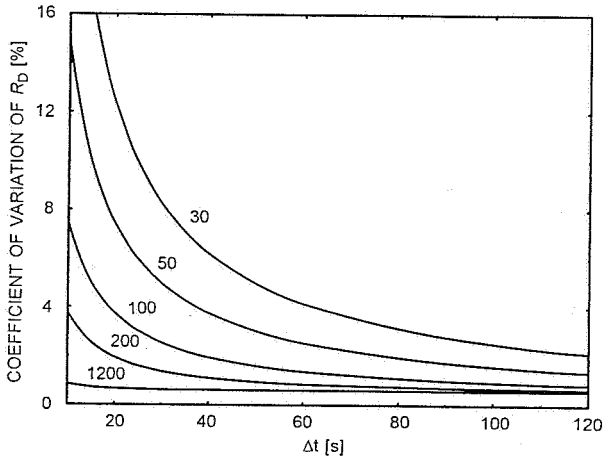

Fig. 4. Error analysis showing the coefficient of variation rate (R_D) as a function of the measurement interval (Δt) and the respiration rate (R_D). The values used for each of the contributing variances shown in Eq. 2 are given in the text.

Comparison of magnitude of the variances contributing to the total estimated variance for R_D (Eq. 2) showed that under normal operating conditions (Table 2)

variation in the CO_2 measurement (ΔC) was dominant (Table 3). As ΔC increased with increasing R_D, differences between the assumed and actual temperature and pressure became relatively more important through their effects on V_M. The error in the duration of the measurement (Δt) was insignificant.

Systematic errors: The effects of the systematic errors that we considered to be the most significant were minimised. As mentioned earlier, some contamination of samples did appear to occur when the seals on the 'gas-tight' syringes were inadequate. 'Zero' checks were conducted regularly to correct for this. Analyser calibration was checked several times a day to allow for any analyser drift. A nearly continuous check on the analyser performance was made by monitoring the signal between measurements when only 'flushing' gas, which was of unknown concentration, as flowing through the analyser.

Table 3. Relative importance of the variances contributing to the total estimated variance for respiration rates (R_D) for several time intervals (Δt). The Δt used in practice was selected to maintain the ΔC between 20 and 100 μmol mol^{-1}. For explanation of symbols see Eq. 1.

Measurement interval, Δt, if R_D = 50 pmol s^{-1} [s]	CO_2 increase, ΔC [μmol mol^{-1}]	Portion of the total variance attributed to each of the contributing variances by the error analysis			
		ΔC	Δt	v	V_M
10	2.0	99.8	0.0	0.0	0.2
30	6.1	98.6	0.0	0.1	1.3
60	12.2	94.6	0.0	0.3	5.1
90	18.2	88.7	0.0	0.6	10.7
120	24.3	81.5	0.0	1.0	17.5

Example of use

The temperature responses of kiwifruit buds (*Actinidia deliciosa* cv. Hayward (A.Chev) C.F.Liang *et* A.R.Ferguson) was measured on canes which had been grown under contrasting conditions. One set of five canes, approximately 1.5 to 2.0 m in length, were removed from vines growing in Kerikeri (35°14'S) which is located in the warmest commercial kiwifruit growing region in New Zealand. A second set of canes came from Riwaka (41°06'S) which is located in the coolest commercial kiwifruit growing region. The canes were cut, each from a different vine, on August 11, 1992 which is early spring and approximately 5 to 9 weeks before the normal time of bud break at Riwaka and Kerikeri, respectively (McPherson *et al.* 1994). They were then transported to the laboratory and maintained at a 20 °C air temperature for 24 h. At the end of the experiment, individual buds were removed from the bud cover and were found to have an average dry mass of 0.03 g.

On August 13, two days after they were removed from the vine, the R_D of the dormant buds were measured under a range of temperatures. Measurements of R_D commenced about one hour after each change in temperature as earlier measurements had shown that the temperature of the buds and their R_D stabilised well within this period. The apical (tip) bud on each cane was chosen for measurement because they

normally develop earliest and produce the most flowers (McPherson *et al.* 1992).

Although the sample of buds used was small, some interesting responses were obtained (Fig. 5). All buds showed increasing R_D with increasing temperature in the range 15 to 25 °C. The R_D of dormant buds which had grown in Kerikeri, ranged from 22 to 130 pmol s^{-1} depending on the particular bud and the temperature during the measurement. The buds which had grown in Riwaka ranged up to much higher rates (36, to 1170 s^{-1}). The most likely explanation for the higher R_D of buds from Riwaka is that they were, on average, developmentally more advanced. McPherson *et al.* (1994) found over a three year period, that the date of 50 % bud break was 25 d earlier on average in Riwaka than at Kerikeri. There was high internal consistency in the measurements, the ranking of the buds being similar across the three measurement temperatures.

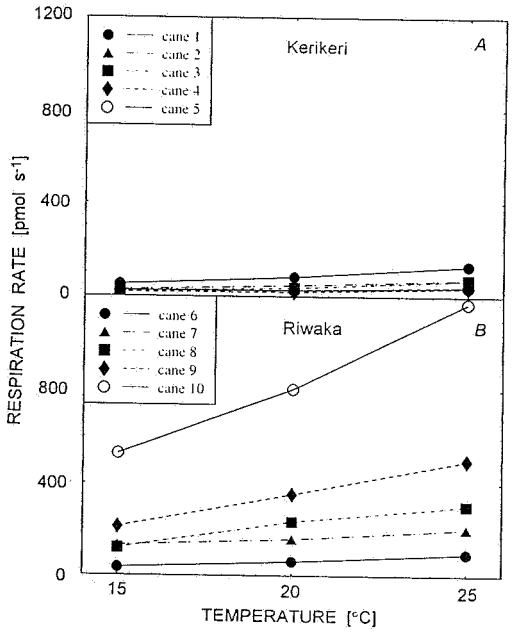

Fig. 5. The response to temperature of the respiration rates R_D of the dormant apical buds of five kiwifruit canes sampled in early spring (August 11, 1992). The canes were sampled from five separate vines which were growing at: (*A*) Kerikeri, in the northernmost commercial growing area in New Zealand, (*B*) Riwaka, the southernmost area.

Conclusions

The technique was found to be reliable even at low rates of respiration and gave consistent measurements on individual buds. The gas-tight syringes proved to be less reliable than expected. An improvement in their quality would be an advantage. The ability to make non-destructive measurements is a considerable advantage because it allows the progress of individual buds to be monitored over an extended period. With two operators, measurements could be made at a rate of one bud every four minutes.

The technique is suitable for use in either the laboratory or the field. The system could be readily adapted to any species where gas-tight seals can be established on individual buds.

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