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Investigation of gas composition (CO2, N2, O2 mixture) on dormancy of potato tuber

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ABSTRACT

Background: For many years ethylene has been known to regulate plant growth and development. This response is also induced by other compounds such as propene, 1-butene, carbon monoxide, acetylene, and isocyanides The possible roles of oxygen and carbon dioxide treatments in the presence or absence of ethylene on tuber dormancy release in potato (Solanum tuberosum L**.**) **Objective :1)** examined the relationship between high O2 and CO2 treatment levels on tuber dormancy release and sprout growth in terms of previous storage temperature and tuber age and, **2)** evaluated the hypothesis that the CO2-O2 action on dormancy release and sprout growth of potato tubers is due to an effect on ABA and sugar levels[3]. **Results :** were examined. Using two gas compositions (I : 60% CO2-20% O2-20%N2 and II : 20% CO2-40% O2-40% N2), the phase of tuber dormancy and previous storage temperature were demonstrated to be important parameters for dormancy release by these gas mixtures. Gas I caused decreased abscisic acid (ABA) levels within 24 h regardless of previous storage temperature, although this effect was reversible. Exogenous ethylene, an effective dormancy release agent, also caused decreased ABA levels within 18 h. Gas II treatment led to slight reductions in ABA levels that were further decreased by ethylene[1].**Conclusion:** The response shows a lag of 8 hours, and more than 24 hours of exposure is required for the maximum effect. The optimum temperature is near 25 °C. Rapid generation of reactive oxygen species (ROS) at the cell surface has been implicated in plant defense responses.

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INTRODUCTION

For many years ethylene has been known to regulate plant growth and development. This response is also induced by other compounds such as propene, 1-butene, carbon monoxide, acetylene, and isocyanides. Dormancy release in buds and seeds by high concentrations of carbon dioxide and oxygen has been observed repeatedly, although the specific physiological mechanisms are unknown (Esashi, Y., 1991). In potatoes (Solanum tuberosum L.), Thornton (1933) initially observed that tuber dormancy could be broken effectively with 40-60% CO2 and 20% O2 applied to tubers continuously for 3-7 d at 25 °C. He subsequently demonstrated an enhancement of this effect by high (20-80%) concentrations of O2 (Thornton, 1939). He hypothesized that normal termination of tuber dormancy was due to a relatively O2-impermeable periderm while CO2 acted as a `metabolic regulator'. Subsequent work, however, did not support the role of O2 as the major and sole factor that regulated dormancy (Sawyer and Smith, 1955).

Consequently, the present study 1) examined the relationship between high O2 and CO2 treatment levels on tuber dormancy release and sprout growth in terms of previous storage temperature and tuber age; and, 2) evaluated the hypothesis that the CO2-O2 action on dormancy release and sprout growth of potato tubers is due to an effect on ABA and sugar levels (Smith, J.J., P. John, 1993).

In addition to its dormancy breaking effect, ethylene also influences the number of sites of sprouting of tubers, indicating an effect on apical dominance. Increased sprout numbers per tuber, following ethylene exposure, are reported by Wills and Warton (2003), Prange *et al.*(2005a and 1998) and Kalt *et al*. (1999), though these are reporting on ware potato storage trials.

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Ethylene is a plant hormone having diverse effects on a wide range of plant tissues. In spite of its apparent importance a regulator of plant growth, the site and mechanisms of its actions have not yet been elucidated (Streck, N.A *et al*., 2006; REID, M.S., and H.K. PRATT, 1970; Patel, N., T.B.S. Rajput, 2006). Ethylene evolution is observed under many situations, including mechanical wounding, hypoxia, environmental pollution, and invasion by pathogens.

MATERIALS AND METHODS

Tuber production & experiments:

Tubers of the cultivar **"satina"** in Ardabil during the summers of 2009,2010 and 2011. Plants were top killed after 90 d and dug 4weeks later, in accordance with normal seed production practices. Tuber periderm matured at 15-17 °C for 13-15 d followed by storage at 5 or 15 °C until required.

Uniform tubers (70-120 g f.wt per tuber; 25-45 tubersper treatment) were treated in the sample chambers for periods of 2-8 d at 20-25 °C with a range of gas mixtures composed of nitrogen, carbon dioxide, oxygen and}or ethylene. Untreated tubers and tubers treated for 18 h at 20-25 °C with bromoethane (BE) vapour, an effective dormancy release agent (0-20 ml liquid per litre of treatment chamber; Coleman, 1983) served as reference controls. After treatment, tubers were either placed in controlled environmental facilities (20 °C constant dark), or planted directly in sterile loam (approx. 2 cm soil covering), under glasshouse conditions, with supplemental fluorescent and incandescent lighting (18 h photoperiod) and variable temperature conditions (20-25 °C day and 14-17 °C night) (Coleman, W.K., J. McInerney, 1997; Qin, L., *et al*., 2004).

Dormancy release and associated sprout growth:

Tuber dormancy release and sprout emergence from the soil surface were recorded two±three times per week using d after planting the `mother' tuber (DAP) as a time basis (Cho, Iritani and Martin, 1983). In the present study, three reference stages were distinguished: an early initial growth stage that was characterized in this study by a 1 mm reference threshold, an intermediate stage of linear growth rate (3 mm reference) and a fnal stage of exponential sprout growth rate (10 mm reference). `Phases' of the dormancy period were defined in terms ofcultivar and DAP. For example, Russet Burbank seed tubers progressed through an arbitrarily defined early (80-150 DAP) and late (150-220 DAP) dormancy phase.Dormancy release would normally occur in untreated tubers during the latter part of the late phase. (Cosgrove, M.S., *et al*., 1998)

Results:

Effect of Ethylene Treatment on Sprout Growth:

The effects of two concentrations of ethylene on growth of the sprouts is shown in Table 1. The elongation rate was inhibited in ethyltreated sprouts in both concentrations in comparison to the control (Rylski, I., *et al*., 1974). The extended treatment caused greater inhibition of sprout elongation; the shorter, ethylene-treated sprouts were thicker than the controls, but the weight of the sprouts in all treatments was similar.

Effect of tuber age and storage temperature:

Tubers that were treated at 118 DAP during the early dormancy phase (70-140 DAP) were quite sensitive to the previous storage conditions in terms of responsiveness to

gas mixtures I and II. Tubers removed from a 5 °C storage responded to gas II regardless of the reference length chosen. However, when tubers were removed from a 15 °C storage, they were not affected by this treatment (Table 2).When tubers were treated with gas I, an opposite response occurred. While tubers from 5 °C responded significantly at the 5 and 12 mm reference lengths, but not at all at the 1 mm reference length, tubers from 15 °C storage were very responsive to gas I in terms of reduced dormancy duration. Tubers treated at 157 DAP during the late dormancy phase (150-220 DAP) demonstrated that both CO2- O2 mixtures significantly reduced dormancy using the 1 and 3 mm reference lengths (Table 2). However, gas I treatment was more effective at the 12 mm reference length than gas II and this enhanced response was reflected in a more rapid emergence rate (data not shown) (Cvikrova, M., *et al*., 1994)

Subsequent sprout growth from tubers treated during the early dormancy phase was sensitive to previous storage conditions with maximum growth rate exhibited by gas Itreatedtubers after 5 °C storage (Fig. 2). The differential response of dormant tubers to the gas mixtures during the early dormant phase was also observed in sugar changes. Gas I-treated tubers previously stored at 5 or 15 °C and treated during the early dormancy phase (129 DAP) led to increased levels of sucrose, glucose and

fructose at the end of the 8 d period when compared to untreated (control) or gas II-treated tubers (Table 2) (Coleman, W.K., J. McInerney, 1997; Qin, L., *et al*., 2004; Emilsson, B., H. Lindblom, 1963). This effect was more pronounced after 17 d. Removal of tubers after 120 h treatment with gas I led to rapid increases (within 24 h) in sucrose concentrations in the apical region (Fig. 1). After a 24 h lag, sucrose levels also increased in thebasal region of the treated tubers.

The Recovery of Tubers from an Initial Ethylene Treatment:

The respiration rate of a large lot of potatoes treated with ethylene for 1 day and then returned to air.

The respiration rate of the treated tubers returned to that of the control by about 4 to 5 days after the removal of ethylene. The respiration rate of individual tubers taken at intervals from the above lot and retreated with ethylene is shown also. Renewed application of ethylene slightly stimulated respiration of once-treated tubers as little as 3 days after cessation of the initial treatment (Van Ittersum, M.K., *et al*., 1992). The respiration response to the second treatment gradually increased with extension of the recovery period, reaching about 67% of the initial response 21days after termination of the first treatment.

The Effect of Ethylene Treatment on the The respiration of potato tubers:

The respiration pattern of potato tubers treated for different lengths of time with 15 µl/l ethylene is shown in Figure 3. After a time lag of about 12hr, the respiration rate

of treated tubers increased rapidly, reaching a peak 18 to 24 hr after the start of the experiment. The peak rate shown by continuously treated tubers was about eight times the initial respiration rate, whereas that of untreated tubers showed littleor no change. Short treatment times were insufficient to bring about such a large effect, although treatment for as little as 6 hr induced a slight increase in respiration over that of the

control tubers. The full effect of ethylene was seen only intubers treated for more than 48 hr (Van Ittersum, M.K., *et al*., 1992; Turnbull, C.G.N., D.E. Hanke, 1985b).

Discussion:

Although a 2-3 mm sprout length from tuber buds has traditionally been used as the criterion for dormancy release (Emilsson, 1949; Reust, 1986; Van Ittersum, 1992), previous studies have noted the discontinuous nature of early tuber sprout growth (Goodwin, 1966). In this context, emergence was viewed as an integration of dormancy release and sprout growth effects. Similarly, the concept of two dormancy phases of approximately equal duration can be found in the work of Macdonald and

Osborne (1988): an early phase of low levels of nucleic acid and protein synthesis, and a late cell expansion phase (`white tip ' phase). Differences in effectiveness of gas I compared to gas II in terms of dormancy release and subsequent sprout growth must be considered primarily to be due to the complex effects of, inter alia, previous storage temperature, tuber age and dormancy release stage (Qin, L., *et al*., 2004; Cosgrove, M.S., *et al*., 1998; Rylski, I., *et al*., 1994). For example, gas II treatment of tubers from the early dormancy phase was effective from 5 °C storage, but ineffective when removed from 15 °C storage, regardless of reference length. However, gas II was effective at 1 and 3 mm sprout reference lengths when removed from 5 or 15 °C storage during the late dormancy phase (Table 1). The current study links specific CO2-O2 treatments and decreased ABA levels. This link may be interpreted in the context of earlier hypotheses of a causal connection between dormancy release, sprout growth and an endogenous inhibitor containing ABA (Burton, 1958; Goodwin, 1966; Hemberg, 1985). The CO2-O2 treatments were effective in reducing ABA levels in dormant tubers, decreasing tuber dormancy duration and increasing sprout growth rate. Previous work has indicated a significant negative correlation between sprout growth rate and initial ABA levels in tuber tissue of ten potato cultivars (Coleman and King, 1984). Exogenous ABA is also capable of inhibiting potato sprout growth when applied repeatedly at high concentrations (El-Antably, Wareing and Hillman, 1967). Studies have implicated ABA and cytokinins in tuber dormancy and sprout growth control with less well defined roles for GA and ethylene (El-Antably *et al*., 1967; Bailey, Phillips and Pitt, 1978; Van Staden and Dimalla, 1978; Turnbull and Hanke, 1985a, b; Cvikrova *et al*., 1994) and no direct role for IAA (Sukhova *et al*., 1993) (REID, M.S., and H.K. PRATT, 1970). The multiple aspects, quantitative features and dynamic nature of a control system with possible feedback interactions suggests that a conceptual model using a dynamic systems approach (Trewavas, 1986) may be helpful in further delineating dormancy control in potato tubers by endogenous plant growth regulators, as well as such exogenous agents as ethylene, CO2 and O2. The present study has demonstrated that CO2-O2 mixtures and, to a lesser extent, exogenously applied ethylene can modify sugar levels, reduce ABA levels or reduce dormancy duration in potato tubers.

Table 1: Effect of Differing Concentrations anid Durationt of Ethylenze Treatment on 'Ardabil ' PotatoSprout Growth.

Treatment	Sprouts	
	Growth in length	Final weight
	mm	mg
Control	25.5	652
2µl/l – 48 hr	8.5	609
20µl/l – 48 hr	7.8	528
2µl/l – 12 day	5.2	638
20µl/l – 12 day	4.8	588

Journal of Applied Science and Agriculture, 9(6) May 2014, Pages: 2351-2355

Table 2: Effect of gas composition and pre.ious storage temperatures on sugar levels in apical eye tissues of Ardabil tubers from the early dormancy phase (70-140 DAP) Control, Ambient atmosphere of 0.03% CO2 and 20.9% O2; gas I, 60%CO2- 20%O2 ; gas II, 20%CO2- 40%O2. Treatments applied for 8 d starting at zero (129 DAP).

Treatment	Day	Sucrose	Glucose	Fructose
		µmol g−1 d wt (+s.e.)		
5 °C storage Control	0	236±4 (3±8)	202±3 (6±0)	198±7 (5±9)
	8	48±7 (0±1)	124±0 (0±3)	75±9 (0±4)
	14	32±7 (0±1)	120±6 (1±4)	66±5 (0±1)
Gas I	0	-	-	- -
	8	219±1 (0±7)	223±5 (0±4)	212±2 (0±0)
	14	155±9 (0±4)	173±2 (0±4)	193±4 (1±3)
Gas II	0	-	-	- -
	8	173±8 (0±4)	92±7 (0±5)	70±0 (0±6)
	14	57±9 (0±2)	78±2 (0±5)	52±9 (0±2)
15 °C storage Control	0	14±3 (0±3)	8±6 (0±3)	3±4 (0±3)
	8	15±0 (0±3)	10±9 (0±1)	5±7 (0±1)
	14	14±1 (0±3)	3±2 (0±7)	4±1 (0±1)
Gas I	0	-	-	- -
	8	75±0 (0±0)	15±2 (0±0)	12±9 (0±1)
	14	187±6 (0±4)	119±2 (0±1)	97±2 (2±7)
Gas II	0	-	-	- -
	8	20±7 (0±1)	4±2 (0±3)	3±9 (0±2)
	14	21±1 (0±4)	7±1 (0±1)	3±0 (0±0)

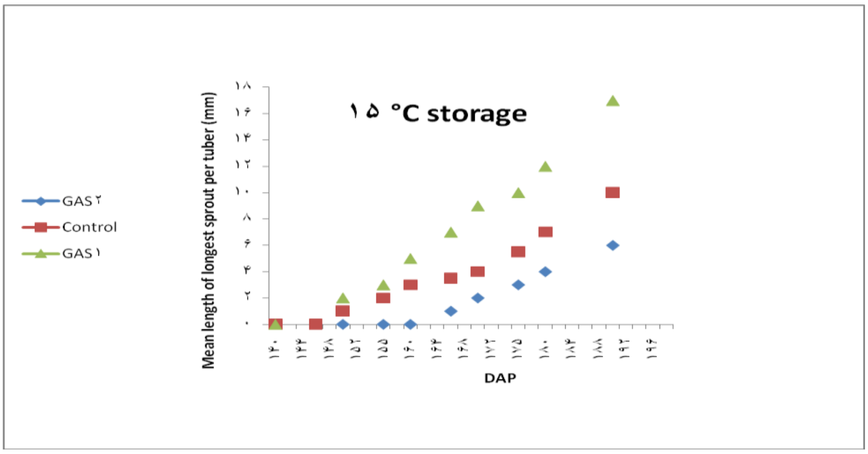

Fig. 1: Sprout growth from Russet Burbank tubers from the early dormancy phase (70-140 DAP) removed from 15 °C storage and treated for 8 d with gas I (60%CO2- 20%O2), gas II (20%CO2- 40%O2) or untreated control (0.03% CO2 and 20.9% O2). Time zero for the different treatments was 129 DAP.

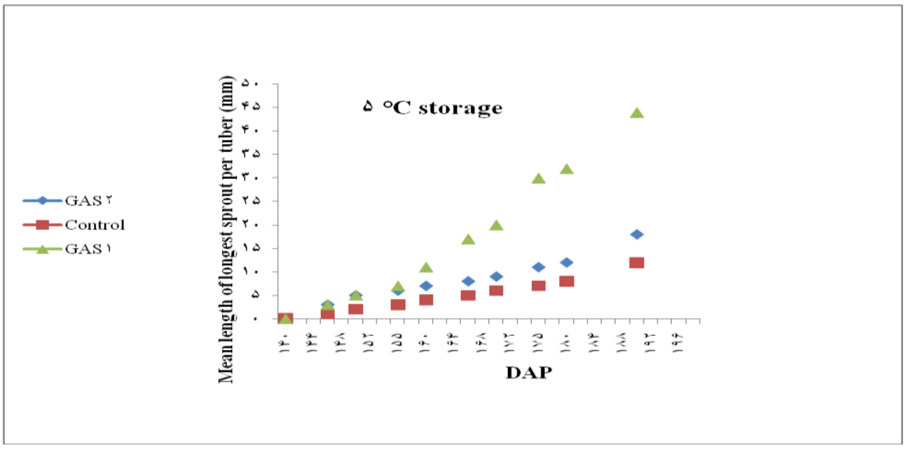

Fig. 2: Sprout growth from Russet Burbank tubers from the early dormancy phase (70-140 DAP) removed from 5 °C storage and treated for 8 d with gas I (60%CO2- 20%O2), gas II (20%CO2- 40%O2) or untreated control (0.03% CO2 and 20.9% O2). Time zero for the different treatments was 129 DAP.

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