Pak. J. Bot., 42(3) : 1761-1770,_2010.

ESTIMATION OF CHILLING REQUIREMENT AND EFFECT OF HYDROGEN CYANAMIDE ON BUDBREAK AND FRUIT CHARACTERISTICS OF 'SUPERIOR SEEDLESS' TABLE GRAPE CULTIVATED IN A MILD WINTER CLIMATE

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Abstract

The chilling requirement and optimum time for hydrogen cyanamide (HC) application were determined for Superior Seedless table grape grown in southern Tunisia, an arid mild winter region. The reliability of five models to predict chilling accumulation for this cultivar was also investigated. In mid-November, current season shoots were excised and subjected to artificial chilling at 7°C for different lengths of time. Each time, half the shoots were treated with a 2% (v/v) aqueous solution of HC, the others were sprayed with distilled water. Thereafter, these shoots were forced to budburst. Rest intensity gradually declined due to chilling accumulation. We estimated that the cultivar needed approx. 440 hours (h) of chilling, or chilling requirement (CR), to overcome endodormancy. During two dormant seasons, estimation of chilling accumulation showed that the Positive Chill Unit model was the most suitable to predict rest completion for Superior Seedless grown under our climatic conditions. Using this model, we found that the variety's CR was not always met by mid-February. In both laboratory and field trials, HC was most effective in enhancing and advancing budbreak if applied when approx. 2/3 of the cultivar's CR were met. Moreover, by this application berry weight and diameter were increased and fruit maturity was advanced. Our study indicated that HC (2%) was effective in advancing budbreak and fruit maturity of Superior Seedless table grape although its effectiveness depended on application date.

Introduction

Most temperate zone perennials undergo a yearly period of bud dormancy which is an adaptive response to survive unfavorable winter conditions. Lang *et al*., (1987) designated three successive phases of bud dormancy as follow: paradormancy is regulated by physiological factors within the plant but outside the dormant structure; endodormancy, coinciding with winter, is regulated by physiological factors within the bud and is released by chilling temperatures (Balandier *et al.*, 1993; Shirazi, 2003). Finally, ecodormancy is an inhibition imposed by environmental factors after endodormancy release; it ends when warm temperatures cause ecodormant buds to burst. Therefore, buds of most deciduous trees must receive an amount of chilling which varies among cultivars to resume growth. In mild-winter regions, chilling insufficiency prolongs dormancy and causes abnormal patterns in budbreak and development resulting in a lower commercial production (Nir & Lavee, 1993; Mohamed, 2008). Therefore, application of artificial rest breaking agents such as hydrogen cyanamide (HC) which is the most useful for grapevines (Trejo-Martínez *et al.*, 2009) is needed. Such treatments improve budbreak and productivity of kiwifruit (Powell *et al.*, 2000). However, the effectiveness of the treatment depends on the time of application. Hence, the need to know the cultivar's chilling requirement and chill unit (CU) accumulation. Under field

conditions, air temperature fluctuations and other factors make chilling requirement determination imprecise (Dennis, 2003). For this reason, several models were proposed to estimate chilling accumulation and predict endodormancy release. The success of these models varies from one region to another.

The aims of this work were to determine the chilling needs for dormancy release of Superior Seedless grapevine, to investigate the suitability of five models to quantify chilling accumulation under south Tunisia conditions and to estimate the proper time to apply hydrogen cyanamide in order to improve budbreak and fruit characteristics of this cultivar.

Materials and Methods

Plant material: Six-year old vines of the early ripening table grape *Vitis vinifera* cv. Superior Seedless were used in this trial. They were located in two commercial vineyards near the town of Medenine, Tunisia (33°19' N; 10°23' E; altitude 117 m) where winters are generally mild. The vines were grafted on R110 rootstock, planted with 2 m x 3 m spacing on a sandy soil and trained onto a two-wire trellis. Vines were pruned to four long canes with an average of 16 buds each and four spurs.

Estimation of onset of endodormancy: From the beginning of October 2003 until the end of November, current year canes with an average of eight buds (in positions 4 to 11) were collected each week from several vines. In the laboratory, they were placed with their basal tips in distilled water and forced under forcing conditions according to Or *et al.,* (2002). The canes were considered dormant if no bud has burst after one month of forcing.

Estimation of chilling requirement and time of hydrogen cyanamide application: In mid-November 2003 and 2004, several canes were collected as described above. They were exposed to low temperature in a dark cold room (7°C ± 1) to simulate chilling accumulation. Twelve canes were pulled out of the cold room every 24 h. Each time, six canes were sprayed to run off with an aqueous solution of Dormex® (Degussa AG, Trosberg, Germany), containing 2% (v/v) HC and no surfactant. The other six canes were sprayed with distilled water to serve as controls. The canes were then forced for one month and the percentage of budbreak was determined; buds were considered open when green tissue became visible. We considered chilling requirement has been satisfied when 50% of the buds have broken (Ghariani & Stebbins, 1994).

In field trials, 2% (v/v) HC was applied on the 1st (A), 9th (B) or 18th (C) January 2004. Each treatment was applied to three blocks consisting of three vines at each one of the two vineyards. Similarly, others vines were sprayed with water on the 1st of January to serve as controls. On each treated vine, two canes were marked and used to monitor budbreak. The buds were considered either dormant (D), swollen (S), or open (O). These phenological growth stages were evaluated at two different times: 20 February and 04 March. Final budbreak was quantified at the end of the growth season in one vineyard. For each treatment, random samples of one bunch per vine were taken at the beginning of the commercial harvest (10 July). The berries were weighed and their diameters were measured, then they were crushed and the juice was used to determine total soluble solids (°Brix). To assess maturity progress total soluble solids content was also measured on 20 June.

Table 1. Chill unit factors (CUF) used with Utah (UT), Low Chilling (LC) and North Carolina (NC) models (Adapted from Carla *et al.*, 2004).

Utah model		Low Chilling model		North Carolina model	
Temperature (°C)	C U F	Temperature (°C)	C U F	Temperature (°C)	C U F
<1.5	0	≤1.7	0	≤1.5	0
1.5-2.4	0.5	1.8-7.9	0.5	1.6-7.1	0.5
2.5-9.1	1	8-13.9	1	7.2-12.9	1
9.2-12.4	0.5	14-16.9	0.5	13-16.4	0.5
12.5-15.9	0	17-19.4	0	16.5-18.9	0
16-18	-0.5	19.5-21.4	-0.5	19-20.6	-0.5
>18	-1	≥21.5	-1	20.7-22	-1
				22.1-23.2	-1.5
				≥23.3	-2

Winter chilling computation models: In the present study, we compared five models. The first one and simplest was developed by Weinberger (1950) who defined one chill unit as one hour below 7.2°C. The 'Utah Model' (UT) (Richardson *et al.*, 1974), the 'Low Chilling' model (LC) (Gilreath & Buchanan, 1981) and the 'North Carolina' model (NC) (Shaultout & Unrath, 1983) are defined in Table 1. The fifth model a variation of Richardson model (Positive Chill Unit model; PCU) suggested by Linsley-Noakes *et al*., (1995) who adopted the same unit factors used in UT model but ignored the negative daily totals.

Chilling accumulation was evaluated between 15 November and end of February. Hourly temperatures needed to calculate chill unit accumulation were estimated from daily minimum and maximum temperatures (Fig. 1) using a computer program written for this purpose. According to Linvill (1990), the minimum in daily temperature is reached just prior to sun rise (around 6 a.m) and the maximum daily temperature is reached at midday (around 1 p.m). The program assumes that temperature changes linearly as a function of time between the two extremes. It increases from 6 a.m to 1 p.m of day i and decrease from 1 p.m of day i to 6 a.m of day i+1.

Statistical analysis: Data of bud burst in the field and fruit characteristics were subjected to analyses of variance (ANOVA) for a randomized block design using SAS statistical software version 6.12 (SAS Institute, Cary, NC, U.S.A). Where applicable, means were separated by Duncan's Multiple Range Test. Data from the hydrogen cyanamide application in the laboratory were analyzed using student's T-test with a level of significance p=0.05.

Results

Estimation of the onset of endodormancy: Canes collected on 10 November broke several buds when forced in a growth chamber whereas those collected on 17 November or later did not burst any buds during one month of forcing. Therefore, we considered that buds became dormant in mid-November which coincided with late leaf fall.

Variety chilling requirement estimation: Budbreak percentage increased rapidly when canes were exposed to chilling temperatures before being forced (Table 2). It appears that rest intensity decreases as chilling accumulates. The number of chilling hours at 7°C needed to obtain 50% bud burst was about 440. Therefore, the cultivar seems to require approx. 440 CU to resume normal growth.

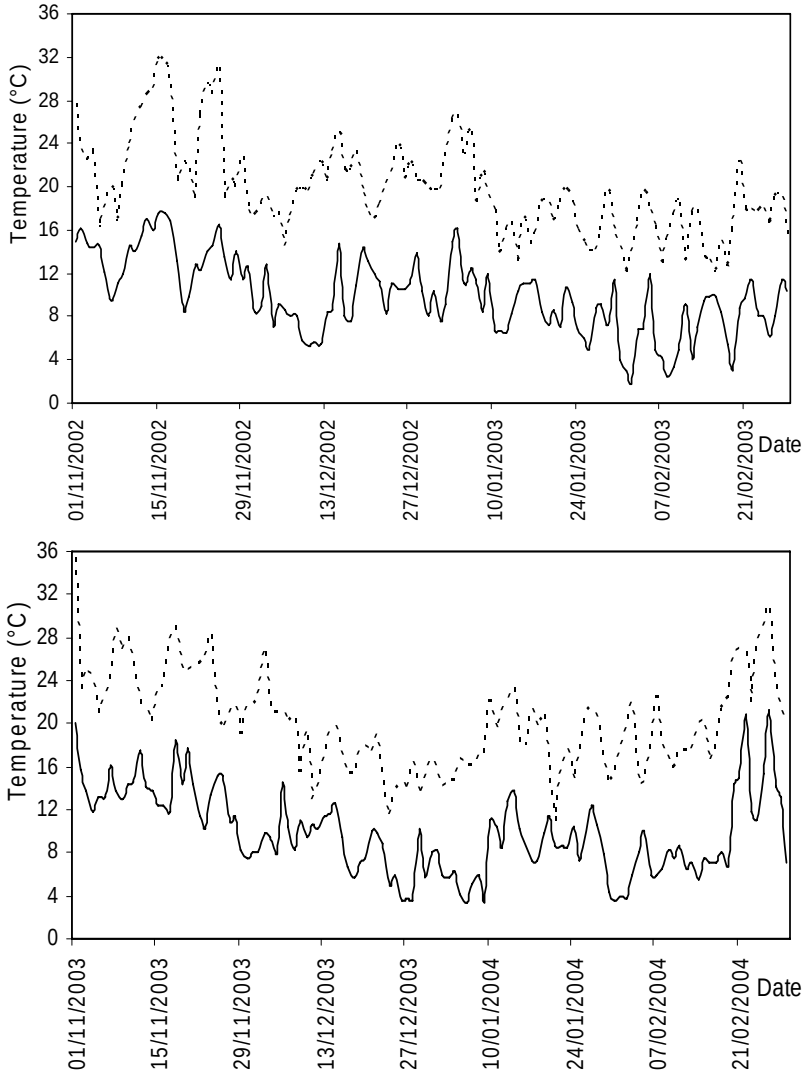

Fig. 1. Maximum and minimum daily temperatures in Medenine during two successive rest seasons.

Efficiency of Hydrogen cyanamide application: In the laboratory trial, application of HC to detached and chilled canes improved budbreak (Table 2). Canes sprayed with HC reached 50% budbreak the fastest when exposed to at least 320 h of cold. Furthermore, HC hastened budbreak when canes were first exposed to 272 to 392 h of cold or the equivalent of 62% to 89% of the cultivar's chilling requirement.

Under field conditions, HC treatments gave comparable results in the two vineyards. Treatment by vineyard interaction was not significant for all measured parameters of budbreak and fruit characteristics. Therefore, data from the two vineyards were pooled.

Table 2. Effect of artificial chilling alone or in combination with a HC application on budbreak of cut vine canes.

Chilling (h)	% of bud burst	
	- HC	+ HC
100	1.2 ± 2.0 a[z]	2.2 ± 2.1 a
200	2.6 ± 2.3 a	6.6 ± 1.5 a
224	5.7 ± 1.9 a	10.0 ± 2.4 a
248	11.9 ± 3.9 a	22.1 ± 3.8 a
272	13.8 ± 2.5 a	29.2 ± 3.8 b
296	24.6 ± 2.1 a	39.9 ± 1.9 b
320	27.3 ± 1.9 a	50.5 ± 2.4 b
344	32.2 ± 4.3 a	54.2 ± 1.6 b
368	33.5 ± 3.5 a	54.6 ± 2.4 b
392	39.6 ± 2.5 a	55.8 ± 2.1 b
416	47.8 ± 4.3 a	56.3 ± 3.7 a
440	49.5 ± 3.4 a	57.7 ± 4.4 a
464	53.2 ± 5.4 a	62.4 ± 3.7 a
488	58.9 ± 4.0 a	67.2 ± 3.8 a
512	62.9 ± 2.9 a	69.1 ± 3.0 a
536	70.2 ± 2.8 a	69.4 ± 2.1 a
560	73.9 ± 3.7 a	70.5 ± 1.9 a
584	72.8 ± 4.1 a	74.5 ± 1.8 a

[z] = Each value is the mean ± SD of 3 replicates. Means within the same line followed by the same letter do not differ significantly at $p \leq 0.05$.

Table 3. Percentage of buds at each of the first three phenological stages at two different dates. The vines were either sprayed with water (Ctr) or with a 2% (v/v) aqueous solution of HC on the 1 (A), 9 (B) or 18 (C) January. Final budbreak (FB) was quantified at the end of the growing season just before pruning.

Treatment	20 February			04 March			FB
	D	S	O	D	S	O	
Ctr	71.6a[z]	24.7d	3.7c	14.3a	61.7a	24.0d	80.2a
A	35.7b	54.2a	10.1b	10.9ab	52.3b	36.8c	82.0a
B	13.0c	35.2c	51.9a	8.1b	30.7c	61.2b	83.7a
C	0.5d	46.9b	52.6a	0.3c	22.9c	76.8a	88.1a
ANOVA							
Trt	**	**	**	**	**	**	-
Vineyard	NS	NS	NS	NS	NS	NS	-
Vineyard*trt	NS	NS	NS	NS	NS	NS	-

[z] = Data from the two vineyards were pooled because treatment*vineyards interaction was insignificant. Means within the same column followed by the same letter do not differ significantly at $p \leq 0.05$.

NS = Not-significant, ** = High significant.

HC accelerated bud development regardless of its time of application (Table 3). On 20 February, vines treated on 1 (A), 9 (B) or 18 (C) January had 10%, 52% and 53% of their buds open, respectively, whereas, untreated vines had only 4% of their buds open. Treatments B and C gave comparable budbreak percentages on 20 February. Whereas, on 4 March, the latter treatment was the most effective (77% vs 61% budbreak). These two treatments achieved 50% budbreak on 20 February, whereas, untreated vines had less than 24% of their buds open on 04 March. Therefore, application of HC on 9 or 18 January accelerated bud development and advanced budbreak by at least 13 days compared to the control. Furthermore, the later application induced a more homogenous budbreak. On 20 February and 04 March, most buds on vines which received this treatment were mostly at two phenological stages, whereas, buds of others vines were distributed over three different phenological stages. Final budbreak (just before winter pruning) tended (p=0.11) to increase with this HC application (Table 3).

Berry weight and diameter were increased by treatments B and C but only slightly by the earliest application (A) (Table 4). At harvest, the weight of berries from treatment C was, on average, 30% heavier than that of berries of untreated vines. Fruit maturity, evaluated by total soluble solids content (°Brix), was advanced by HC when sprayed on 9 (B) or 18 (C) January. On 20 June, fruit from treatments B and C had higher soluble solids contents than those from treatment A and the control. At the second sampling (10 July), we estimated that the vines which received treatment C reached nursery recommended commercial maturity (at approx. 15.5 °Brix) at least one week before those left untreated.

Chilling accumulation assessment: The total number of hours below 7.2°C reached 116 and 130 by mid-February in 2003 and 2004, respectively (Table 5). This represents less than 30% of the cultivar's chilling requirement as determined in the laboratory. The LC model gave negative accumulations during November then more 1000 CU by mid-February. The NC model gave negative accumulations until 10 December then reached more than 760 and 960 units by mid-February of 2003 and 2004, respectively. These last two models gave high CU totals, nearly twice the variety's CR. Utah model, gave negative accumulations for most of the season. If we consider only the PCU, effective chilling temperatures were recorded only from early December to mid- February. By this date, the total accumulation for this region would be 325 CU and 443 CU in 2003 and 2004, respectively. The cultivar's CR was achieved by mid-February in 2004; whereas, only 74% of this requirement was met in 2003.

Discussion

Grape bud dormancy release requires sufficient exposure to proper chilling temperature. In mild winter climates, this requirement is generally only partially met. Therefore, not all buds are able to break and even when they break they often give shoots of low vigour (Erez, 1987)

In our region, the vines became dormant by mid-November, and chilling accumulation was counted from 15 November to the end of February. This is in agreement with previous studies carried out in other temperate fruit cultivars and which consider leaf fall as the start of endodormancy (Gariglio *et al.*, 2006) and useful chilling accumulation occurred from that time until mid-February (Ruiz *et al.*, 2007).

Table 4. Effect of HC application on berry weight (g), diameter (cm) and total soluble solids content (°Brix). Berries were collected from vines sprayed with water (Ctr) or with a 2% (v/v) aqueous solution of HC on the 1 (A), 9 (B) or 18 (C) January.

Treatment	Berry weight	Berry size	°Brix	
	10 July	10 July	20 June	10 July
Ctr	4.37c[z]	1.66b	10.8b	14.6b
A	4.86bc	1.73ab	11.2b	15.1b
B	5.28ab	1.75a	12.8a	15.6ab
C	5.69a	1.81a	13.2a	16.3a
ANOVA				
Trt	**	*	**	*
Vineyard	NS	NS	NS	NS
Vineyard*trt	NS	NS	NS	NS

[z] = Data from the two vineyards were pooled because treatment *vineyards interaction was insignificant. Means within the same column followed by the same letter do not differ significantly at $p \leq 0.05$.
NS = Not significant, ** = High significant, * = Significant.

Table 5. Chilling unit accumulation during two dormant seasons (2002-03 and 2003-04) in calculated using five different models at Medenine (Southern Tunisia).

Date	Hours below 7.2°C		Low chilling model		North carolina model		Utah model		PCU model*	
	02/03	03/04	02/03	03/04	02/03	03/04	02/03	03/04	02/03	03/04
15 Nov.	0.0	0.0	-19.0	-0.5	-35.0	-4.0	-23.5	-13.5	0.0	0.0
25 Nov.	0.0	0.0	-69.5	-69.5	-164.5	-156.0	-181.0	-186.5	0.0	0.0
05 Dec.	1.0	0.0	50.5	18.0	-68.5	-97.0	-220.0	-245.0	12.5	1.5
15 Dec.	10.0	0.0	167.5	168.0	31.0	24.0	-224.5	-264.0	39.5	19.5
25 Dec.	10.0	19.0	249.0	351.5	94.5	204.5	-287.5	-179.0	39.5	106.0
05 Jan.	10.0	66.0	308.0	568.0	107.5	410.5	-389.5	-46.5	39.5	238.5
15 Jan.	14.0	87.0	461.0	681.5	237.0	504.0	-374.0	-51.0	81.0	280.0
25 Jan.	20.0	88.0	639.5	854.0	400.0	660.5	-331.0	-15.0	128.0	324.0
05 Feb.	58.0	115.0	839.5	1024.5	588.5	811.0	-237.5	32.0	222.0	393.5
15 Feb.	99.0	127.0	1028.5	1188.5	767.5	962.5	-134.0	81.0	325.5	443.0
25 Feb.	116.0	130.0	1206.5	1185.0	931.5	886.5	-71.5	-20.5	404.5	457.5
28 Feb.	116.0	131.0	1253.5	1181.5	971.0	857.5	-73.0	-70.0	407.5	458.5

PCU model* = Positive chill unit model.

In the laboratory, Application of artificial cold at 7°C to dormant canes indicated that the cultivar requires 440 CU to overcome endodormancy. In comparison, Cabernet Sauvignon needs 336 h at 6°C to break dormancy (Botelho *et al.*, 2007).

Hydrogen cyanamide has been used effectively to supplement cold temperature to achieve satisfactory budbreak (Dokoozlian *et al.*, 1995). However, its effectiveness depends on the time of application (Or *et al.*, 1999). We used two sets of trials, in the laboratory and in the field, to determine the proper time to apply HC to Superior Seedless table grapes under southern Tunisia conditions.

Laboratory results show that the effect of HC application depended on the amount of chill units accumulated which should reflect the physiological state of the dormant bud. It appears that buds are not responsive to hydrogen cyanamide until they accumulate approximately. 2/3 of their chilling requirement. This is consistent with previous reports

suggesting that the best time to apply restbreaking agents is when 2/3 to 3/4 of the chilling requirement of temperate perennials buds are met (Erez, 1987; Faust *et al.*, 1997; Powell *et al.*, 2000). Later applications can damage buds (Or *et al.*, 1999) and have a thinning effect.

The results of field applications of a 2% (v/v) aqueous solution of HC at three different times in the 2003/2004 season show that the 18 January application (C) was the most effective in enhancing and advancing budbreak. This application advanced budbreak by at least 13 days. In South Africa, applications of 1.25% (v/v) aqueous solution of HC three and six weeks before natural budbreak improve the percentage of bud opening (Lombard *et al.*, 2006). In Chile, maximum budbreak percentage was reached as much as 40 days earlier with a 2.5% (v/v) HC applications (Perez & Lira, 2005).

Treatment C not only hastened bud development but also induced a more homogenous budburst (buds at fewer phenological stages) in comparison to others treatments. Such effect was previously reported by Carreno *et al.,* (1999). The later HC application advanced fruit ripening too; this makes HC a useful tool for the grower to manage fruit harvest.

Dommerc *et al.,* (1986) reported that HC application increases the number of bunches per shoot. This was not the case in this study (second season data; not shown). HC did increase berry weight and diameter; this could be due to improved shoot growth. Indeed, vines treated with HC tended to have fewer buds that fail to open and less weak shoot; this should increase total leaf area and photosynthetic activity thus the improved berry weight and diameter. Hydrogen Cyanamide may be used to advance and homogenize budbreak leading to better fruit quality (size) and earlier maturity; this should permit higher fruit prices.

Estimates of chilling accumulation from meteorological data of the region differed with the model used for computation. According to all tested models except Utah model, chill unit accumulation during the second half of November is negligible. Most chilling accumulation took place during December, January and the first half of February. Similar results were reported by Ruiz *et al.,* (2007) under the Mediterranean conditions of south-east Spain. During 2002/2003 and 2003/2004 seasons, Weinberger model gave total accumulations far inferior to the cultivar's chilling requirement as determined by laboratory trials. Therefore, this model is not appropriate for our conditions. Similar conclusions were reported by Alburquerque *et al.,* (2008) for southern Spain. On the contrary, the NC and LC models yielded accumulation totals more than twice the chilling requirement of the cultivar; therefore, they were not considered too. The UT model (Richardson model) gave negative accumulations. Thus, it is not satisfactory. Previous reports suggest that this model is not adequate for warm winter regions (Erez *et al.*, 1990) and costal locations (Ghariani & Stebbins, 1994). Since the cultivar is commercially cultivated in our region and gives satisfactory vegetative growth and fruit yield in most years, we can safely assume that the true number of CU accumulated each year should be close to the chilling requirement of the cultivar. Therefore, the PCU gave the best estimates of chilling accumulations in this region. Similarly, this model was reported to be the most adapted to South African warm conditions (Allan *et al.*, 1995; Allan, 2004).

On 18 January, date of the most efficient field treatment, the cultivar received nearly 280 PCU, or the equivalent of 64% of its CR. This result is in agreement with the laboratory trial further indicating that PCU model is most adequate for our region.

In conclusion, the PCU model gave the best estimate of chilling accumulation in our region. This model indicates that the chilling requirement of Superior Seedless grape cultivar is not always met and the use of HC can be beneficial in certain years. Hydrogen Cyanamide application was most effective in overcoming rest and advancing bud burst when approx. 2/3 of the chilling requirement of the cultivar has accumulated. Hydrogen Cyanamide application advanced bud burst, improved berry weight and size and advanced fruit maturity.

Acknowledgements

We are grateful to Degussa, AG (Trosberg, Germany) for providing partial financial support and to Dr. K. Khirouni for helping with the computer program used to estimate hourly temperatures and chill units. The National Meteorological Institute provided temperature data used in this study.

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(Received for publication 23 February 2009)