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THE GERMINATION OF PERENNIAL WALL ROCKET (*DIPLOTAXIS TENUIFOLIA* (L.) DC.) AND ANNUAL GARDEN ROCKET (*ERUCA SATIVA* MILL.) UNDER CONTROLLED TEMPERATURES

ABSTRACT

The germination characteristics of three cultivars of perennial wall rocket and annual garden rocket were assessed at constant and diurnal temperatures for ten days. The seeds were exposed to constant temperatures of 5, 10, 15, 20, 25, 30 and 35°C or diurnal temperatures of 24/10, 28/16 and 18/5°C, with a 12/12 h exposure of each regime. The diurnal temperatures were chosen to reflect the minimum and maximum ambient temperatures experienced in Ellis Lane, Australia; during the spring, summer and winter. The total germination percentage (TGP) of perennial wall rocket was the highest at 20 to 30°C, whereas the highest TGP occurred at 10 to 30°C for annual garden rocket. The daily germination speed (DGS) and germination value (GV) are two useful formulas used to express the germination characteristics of seeds that under certain conditions do not achieve ≥50% germination. For both species, there was a significant interaction between the cultivar and temperature for the DGS and GV at both constant and diurnal temperatures; illustrating that the commercial cultivars of these species germinate at different rates in response to different ambient temperatures. This has important implications for the commercial production of these species, as cultivars with high germination rates need to be chosen for different seasonal conditions. At diurnal temperatures, there was an interaction between the cultivar and temperature for perennial wall rocket, with the cvs. DT1 and DT2 having similar TGPs across all of the temperature regimes, whereas the cv. DT3 had lower TGPs across all of the regimes. There was no interaction between the cultivar and diurnal temperature for annual garden rocket; however, temperature did influence the TGP, with higher values at 24/10 and 18/5°C. The germination of the smaller seeds from the perennial species was found to be more dependent on temperature than the larger seeds from the annual species. This is likely due to differences in stored reserves and the influence of secondary dormancy in the respective species.

Key words: arugula, baby leaf, diurnal, rocket, rugula, salad, season

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INTRODUCTION

Perennial wall rocket (*Diplotaxis tenuifolia* (L.) DC.) and annual garden rocket (*Eruca sativa* Mill.) are native to the Northwestern Mediterranean and have been classified into the Brassicaceae family. These species are utilized around the world in the fresh and processed salad markets. In recent years, many cultivars have been developed to improve the commercial cultivation of these plants; however, very few studies have examined the germination characteristics of these economically important plants.

Germination of Diplotaxis spp.

The germination of *Diplotaxis* spp. can be grouped into those that germinate best at the lower temperature of approximately 15°C, such as *D. virgata* (Cav.) DC. and *D. harra* (Forssk.) Boiss. (Pérez-García *et al.*, 1995; Tlig *et al.*, 2008), and those that germinate best at the higher temperature of approximately 25°C, such as *D. erucoides* (L.) DC. and perennial wall rocket (Kleemann *et al.*, 2007; Pérez-García *et al.*, 1995; Sakcali and Serin, 2009). The optimal temperature for the germination of *Diplotaxis* spp. does not seem to be influenced by the perennial or annual nature of the plants, but rather the environmental conditions under which the respective species have evolved. This is supported by the fact that, under natural conditions, *D. virgata* (Cav.) DC. and *D. harra* (Forssk.) Boiss. germinate during the winter, whereas *D. erucoides* (L.) DC. and perennial wall rocket germinate during the spring and autumn, respectively.

Natural diurnal fluctuations in the ambient temperature have been shown to influence germination in certain plant species (Steckel *et al.*, 2001, 2004; Stoller and Wax, 1973), particularly in those that have smaller seeds such as perennial wall rocket. Such fluctuations are known to break the secondary dormancy of seeds that is related to seasonal temperature cycles (Martínez-Laborde *et al.*, 2007). Pérez-García *et al.* (1995) have shown that *D. virgata* (Cav.) DC. has a higher germination percentage at a diurnal temperature regime of 25/15°C when compared to a constant temperature of 25°C. In contrast, Pérez-García *et al.* (1995) and Martínez-Laborde *et al.* (2007) have shown that diurnal temperatures do not significantly increase germination in *D. erucoides* (L.) DC. However, to the best of the authors' knowledge, no studies have examined the effects of diurnal temperatures on the germination of perennial wall rocket thus far.

Germination of Eruca spp.

When compared to the *Diplotaxis* genus, very few studies have examined the germination characteristics of species pertaining to the *Eruca* genus. Recent studies have focused on alternate seed improvement technologies (Mira *et al.*, 2008; Valbuena and Vera, 2002; Zhang *et al.*, 2005) and their response to other external variables during germination (Faheed, 2005; Moussa, 2006). One of the reasons for this lack in the literature may be because this genus naturally achieves a high level of germination. Pita Villamil *et al.* (2002) have shown that *E. vesicaria* (L.) Cav. achieves germination rates of between 78 and 96% when exposed to temperatures of 10 to 25°C. This study also found that the optimal temperature for germination is between 15 and 25° C, with seeds achieving >87% germination.

Pita Villamil *et al.* (2002) also examined the influence of diurnal temperature on the germination of *E. vesicaria* (L.) Cav. and found no significant difference between germination rates at constant or diurnal temperatures. In another study, Ellis *et al.* (1993) reported that the germination rate of annual garden rocket when exposed to a diurnal regime of 30/20°C was 95%. These studies indicate that some species of the *Eruca* genus germinate with a high level of success over a wide range of temperatures. These responses have not been confirmed in the commercial cultivars of annual garden rocket.

MATERIALS AND METHODS

Two controlled-temperature experiments were conducted under a range of constant and diurnal temperatures to evaluate the germination characteristics of perennial wall rocket and annual garden rocket. Three commercially available cultivars of both perennial wall rocket (European wild rocket, Lefroy Valley [DT1], Apollo, Fairbanks [DT2] and Nature, Seminis [DT3]) and annual garden rocket (Cultivated, Fairbanks [ES4], Highway, Fairbanks [ES5] and Myway, Fairbanks [ES6]) were evaluated. The seeds of these cultivars were obtained directly from the supplier and were stored at 6°C.

General information

There were four replicates of 25 seeds per cultivar for each temperature regime. The experiments were conducted in complete darkness in controlled-temperature chambers (Labec Laboratory Equipment, Australia). The seeds were placed on two sheets of damp filter paper in a glass Petri dish with a diameter of 90 mm; Petri dishes were sealed using Parafilm® to prevent evaporation during the experiment. The seeds were then placed in controlled-temperature chambers, each set at individual constant or diurnal temperatures. The experiments were repeated three times.

Controlled temperature regimes

The germination rates at constant temperatures of 5, 10, 15, 20, 25, 30 and 35°C were evaluated as described above. Three diurnal temperature regimes were used to simulate the seasonal day/night ambient temperature fluctuations in Ellis Lane, New South Wales, Australia, 34.03°S, 150.69°E. Historical temperature data were acquired from the Australian Bureau of Meteorology (Camden airport, station #068192), with temperature regimes based on the latest three-year temperature averages. The following diurnal temperature regimes were used with a 12 h exposure of 24/10, 28/16 and 18/5°C; representing spring, summer and winter, respectively.

Data collection

Germination was considered to have occurred when the radical protruded from the epidermis by ≥2 mm. The individual controlled-temperature experiments were conducted for 10 days, with the germination data recorded daily, enabling the measurement of the total germination percentage and the speed at which germination occurred. The filter paper sheets were kept moist as required after the daily counts.

Germination formulas

The germination characteristics of the seeds were determined by calculating the daily germination speed (DGS) and germination value (GV), as described by Djavanshir and Pourbeik (1976).

Statistical analysis

The data were analyzed using Minitab® 16 (Pennsylvania, United States of America). General analysis of variance (ANOVA) was used to analyze the data, with cultivar and temperature as factors. For single factor significance, differences between means were determined using the least significant difference (LSD, 5%). For a significant interaction between factors, differences between least squares means were determined using the standard error (SE) of the mean. Germination responses of rocket crops were not directly compared, as plants have contrasting reproductive cycles and are from different genera.

RESULTS AND DISSCUTION

The aim of these experiments was to determine the optimal temperature conditions for the germination of perennial wall rocket and annual garden rocket. This information is necessary due to the recent cultivation of these species for the baby leaf salad market. Although these species are collectively referred to as rocket, their germination characteristics under different temperature conditions are distinctively different.

Germination of perennial wall rocket

The germination characteristics of perennial wall rocket were strongly influenced by the temperature for both the constant and diurnal regimes (Table 1). The germination characteristics of the individual cultivars were also significantly different from each other across the temperature regimes, with the exception of the total germination percentage (TGP) at constant temperatures (Table 1). The temperature did, however, have a significant effect on the TGP of perennial wall rocket, which was the highest at 20 to 30°C (Table 2). This result is similar to that reported by Kleemann *et al.* (2007) who found that 30°C was the optimal temperature for the germination of perennial wall rocket. Germination was completely inhibited at the lowest temperature examined, illustrating that the germination of this species is heavily dependent on the temperature. Cultivars of this species germinate with the greatest success at higher temperatures, which is reflective of the natural seasonal temperatures under which germination occurs.

Table 1

Results of ANOVA on the cultivar and temperature regime for the germination characteristics of rocket species and their interaction.

Regime	Factor	TGP	DGS	GV
		Perennial wall rocket		
Constant	Cultivar (C)	ns	**	**
	Temperature (T)	**	**	**
		Interaction		
	C × T	ns	**	*
Diurnal	Cultivar (C)	**	**	**
	Temperature (T)	**	**	**
		Interaction		
	C × T	**	**	**
		Annual garden rocket		
Constant	Cultivar (C)	**	**	*
	Temperature (T)	**	**	**
		Interaction		
	C × T	ns	**	**
Diurnal	Cultivar (C)	*	**	**
	Temperature (T)	*	**	**
		Interaction		
	C × T	ns	**	**

ns, *,** non-significant or significant at $P \leq 0.05$ or $P \leq 0.01$, respectively, ANOVA. TGP, total germination percentage; DGS, daily germination speed; GV, germination value.

At the constant temperatures, there was an interaction between the cultivar and temperature for the DGS and GV of perennial wall rocket (Table 1). At lower temperatures the DGS was similar between cultivars, while at temperatures between 15 to 30°C the cvs. DT1 and DT2 had higher DGSs then the cv. DT3 (Fig. 1). This response to temperature is similar to that of the TGP, indicating that germination is both faster and more complete between these temperatures. Similarly, the cvs. DT1 and DT2 had the highest GVs from 20 to 30°C (Fig. 2). These results indicate that large differences in germination exist in commercial cultivars of perennial wall rocket, and that different cultivars have superior germination at different temperatures. These findings are contrary to those of Kleemann *et al.* (2007) who concluded that there were no differences between the germination of perennial wall rocket seeds collected in the wild. However, Kleemann *et al.* (2007) did not calculate the DGS and GV and thus, may not have identified differences between the germination characteristics of different plant populations. This idea is supported by the lack of any significant difference between the TGPs of cultivars at constant temperatures in the present study (Table 1).

Table 2

Effect of constant temperature on the total germination percentage of perennial wall rocket (PWR) and annual garden rocket (AGR).

Temperature (°C)	PWR	AGR
5	0.2 d[a]	89.0 b[a]
10	24.0 c	95.8 a
15	46.1 b	95.7 a
20	70.4 a	96.4 a
25	81.7 a	95.2 a
30	77.0 a	94.3 a
35	48.1 b	90.3 b
LSD	16.8	3.1

[a]Values in a column followed by the same letter are not significantly different (n = 36), least significant difference 5%.

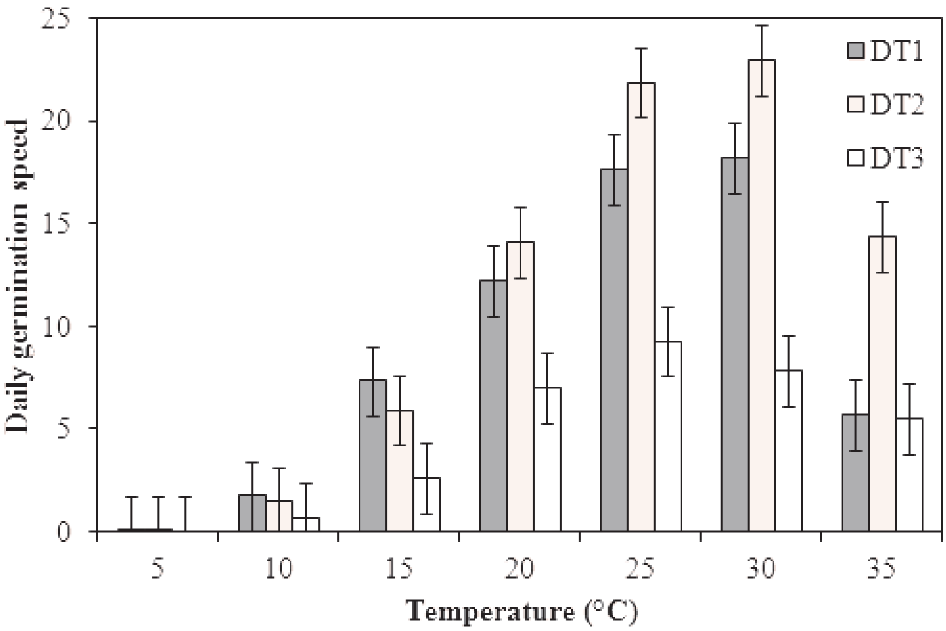

Fig. 1. Interaction effect of cultivar and constant temperature on the daily germination speed of perennial wall rocket; least squares means analysis, n = 12, differences significant at $P \leq 0.01$. DT1, cv. European wild rocket; DT2, cv. Apollo; DT3, cv. Nature.

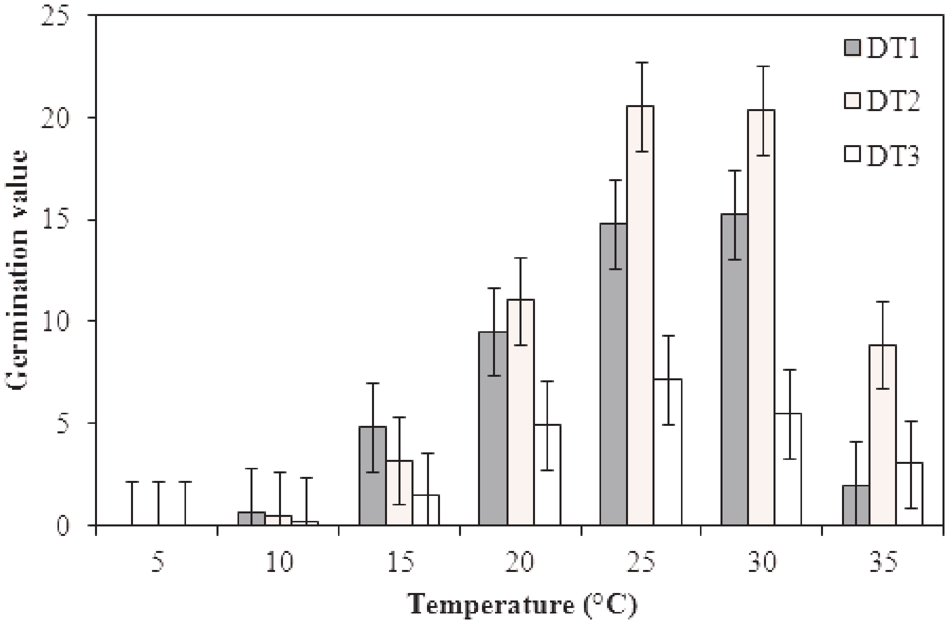

Fig. 2. Interaction effect of cultivar and constant temperature on the germination value of perennial wall rocket; least squares means analysis, n = 12, differences significant at $P \leq 0.05$. DT1, cv. European wild rocket; DT2, cv. Apollo; DT3, cv. Nature.

There was an interaction between the cultivar and temperature for all of the germination characteristics measured at the diurnal temperatures (Table 1). The TGPs of the cvs. DT1 and DT2 were higher than the cv. DT3 at diurnal temperatures of 24/10 and 28/16°C; while at the lowest diurnal temperature regime cv. DT1 had a higher TGP than the other cvs. (Fig. 3). This means that, although cultivars have been selectively bred for commercially desirable traits, there still remains a large difference in their germination responses to temperature. The DGS was the highest for the cv. DT2 at diurnal temperatures of 24/10 and 28/16°C when compared to the other cvs. At the 18/5°C regime the cvs. DT1 and DT2 had higher DGSs than the cv. DT3 (Fig. 4). A different response was shown for the GV, with the highest values for the cvs. DT1 and DT2 across all diurnal regimes (Fig. 5). The diurnal regimes with the highest temperature exposures in this experiment had the fastest and highest level of germination, confirming the importance of higher temperatures in the successful germination of perennial wall rocket seeds.

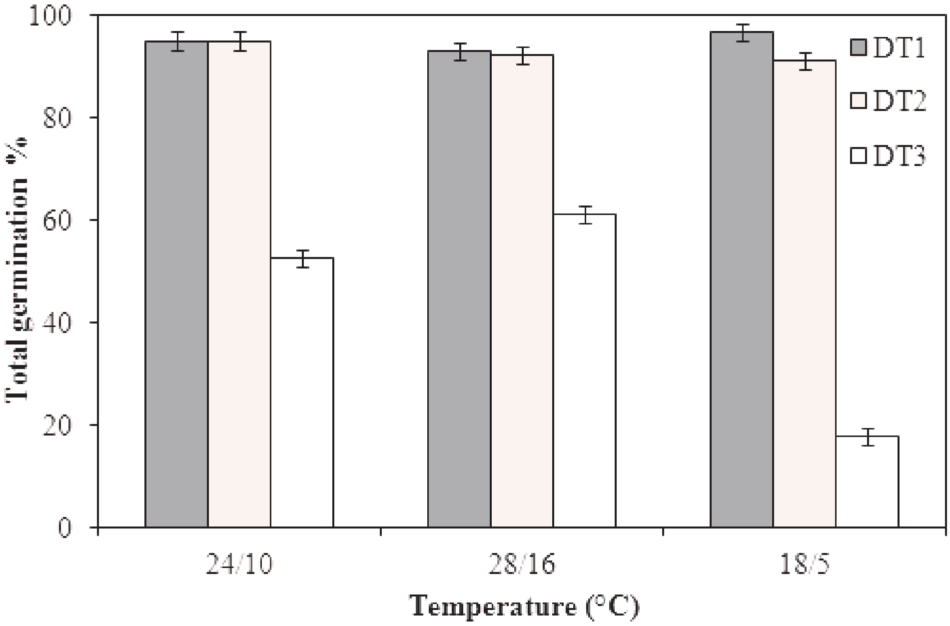

Fig. 3. Interaction effect of cultivar and diurnal temperatures (12/12 h) on the total germination percentage of perennial wall rocket; least squares means analysis, n = 12, differences significant at *P*≤0.01. DT1, cv. European wild rocket; DT2, cv. Apollo; DT3, cv. Nature.

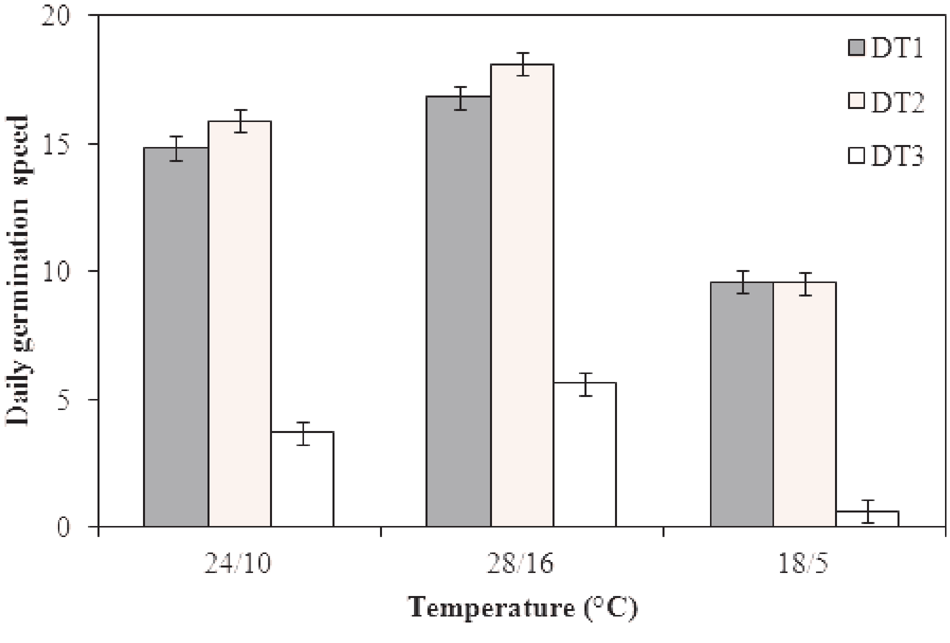

Fig. 4. Interaction effect of cultivar and diurnal temperatures (12/12 h) on the daily germination speed of perennial wall rocket; least squares means analysis, n = 12, differences significant at *P*≤0.01. DT1, cv. European wild rocket; DT2, cv. Apollo; DT3, cv. Nature.

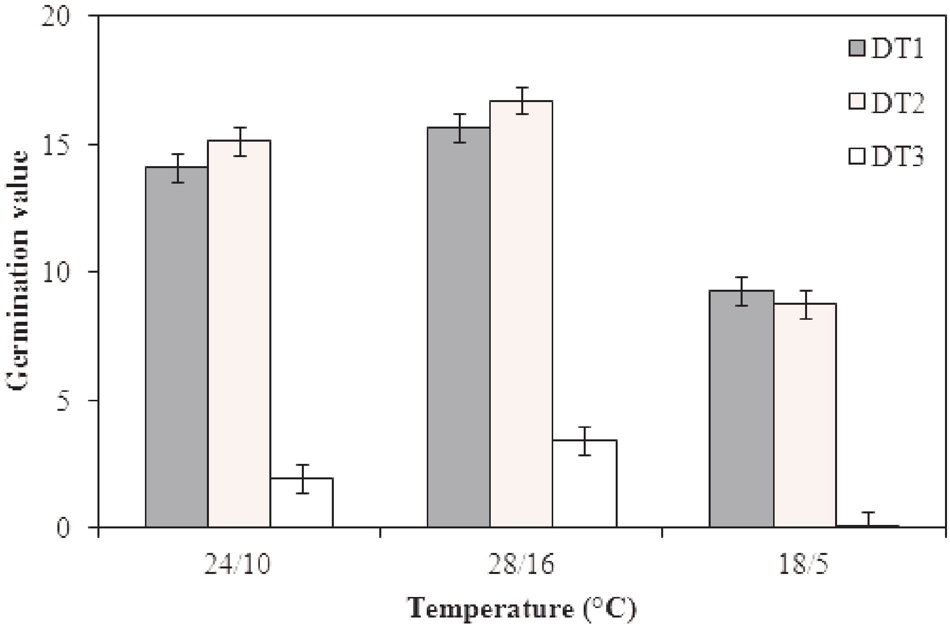

Fig. 5. Interaction effect of cultivar and diurnal temperatures (12/12 h) on the germination value of perennial wall rocket; least squares means analysis, n = 12, differences significant at $P \leq 0.01$. DT1, cv. European wild rocket; DT2, cv. Apollo; DT3, cv. Nature.

D. erucoides (L.) DC. is known to exhibit germination behavior that is similar to perennial wall rocket (Kleemann *et al.*, 2007; Pérez-García *et al.*, 1995). Pérez-García *et al.* (1995) have shown that the TGP of this species was not significantly improved by diurnal temperatures. The results of the current study also indicate that the TGP of perennial wall rocket is not improved by diurnal temperatures. The DGS and GV were also lower under diurnal temperatures when compared to similar constant temperatures, indicating that this natural fluctuation in temperature slows the germination response of this species but does not increase the TGP.

Germination of annual garden rocket

The germination characteristics of annual garden rocket were strongly dependent on the temperature for both the constant and diurnal regimes (Table 1); the individual cultivars also responded differently to constant and diurnal temperatures (Table 1). The interaction between the cultivar and temperature did not influence the TGP of seeds at constant or diurnal temperatures; however, individually these factors had a significant effect on germination. At constant temperatures, the cv. ES4 had higher TGP than the other cvs., whereas the cv. ES4 had higher TGP under diurnal temperatures when compared to the cv. ES5 (Table 3). This means that the different cultivars of this species respond differently to temperature conditions and that care must be taken to choose cultivars with high germination rates. It should be noted that the differences between the germination characteristics of the annual garden rocket cultivars, were much lower than the differences between the perennial wall rocket cultivars. These results support the conclusions of Pita Villamil *et al.* (2002) who found differences in the germination behavior of *E. vesicaria* (L.) Cav. seeds, collected from different wild parental populations.

Table 3

Effect of constant and diurnal temperatures on the total germination percentage of different annual garden rocket cultivars.

Cultivar	Constant	Diurnal
ES4	95.9 a[a]	96.8 a[a]
ES5	92.8 b	93.6 b
ES6	92.9 b	95.4 ab
LSD	2.0	2.2

[a]Values in a column followed by the same letter are not significantly different, least significant difference 5%.

The TGP of annual garden rocket was also influenced by the temperature, with the highest values between 10 and 30°C (Table 2). The TGP of this species over a temperature range of 5 to 35°C was >89%, indicating that, although the temperature does influence germination, moisture is the most important factor that initiates successful germination. These results are similar to those of Pita Villamil *et al.* (2002) who found that the germination of *E. vesicaria* (L.) Cav. was >87% between 15 and 25°C. This means that, under most natural conditions, the presence of sufficient moisture will initiate the germination process.

There was an interaction between the cultivar and temperature for the DGS and GV at the constant temperatures (Table 1). At temperatures of 15° C and below the cvs. ES5 and ES6 had higher DGSs then the cv. ES4; while at 20 to 30°C all cvs. had similar DGSs (Fig. 6). The results for the GV followed a similar trend to the DGS, with higher values for the cvs. ES5 and ES6 at lower temperatures (Fig. 7). These results show that, although the TGP of annual garden rocket was the highest at 10 to 30°C, the optimal temperatures for DGS and GV are from 20 to 30°C. Illustrating that, although the final germination percentage of the seeds is similar outside of this range, the speed at which germination occurs is significantly retarded. This response of seeds to temperature is consistent with the seasonal conditions under which this species naturally germinates.

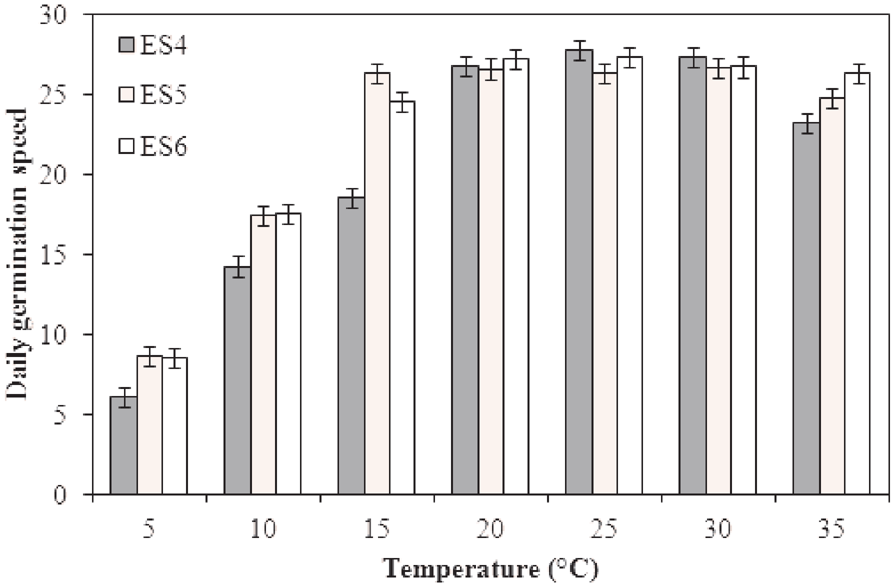

Fig. 6. Interaction effect of cultivar and constant temperature on the daily germination speed of annual garden rocket; least squares means analysis, n = 12, differences significant at $P \leq 0.01$. ES4, cv. Cultivated; ES5, cv. Highway; ES6, cv. Myway.

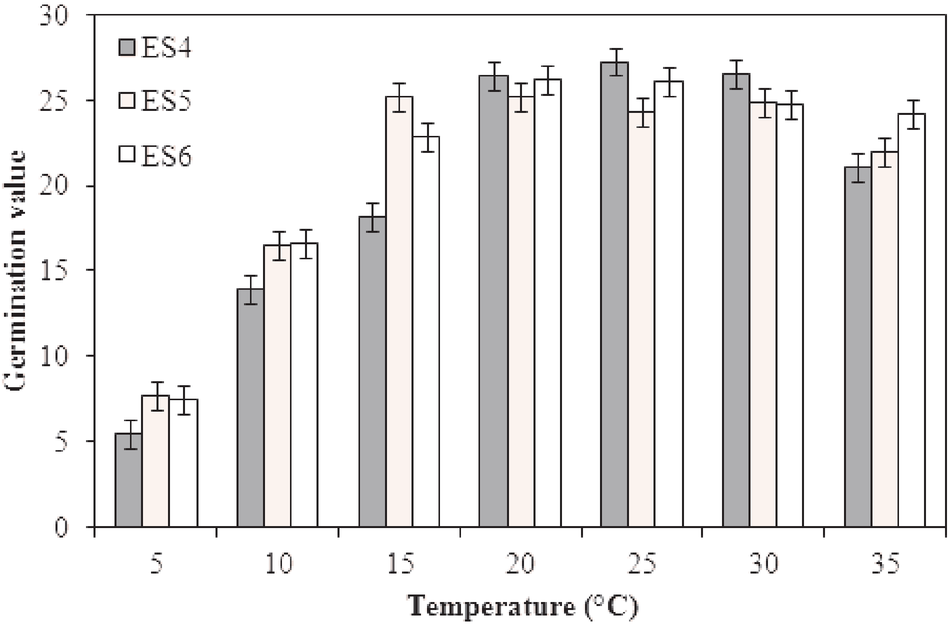

Fig.7. Interaction effect of cultivar and constant temperature on the germination value of annual garden rocket; least squares means analysis, n = 12, differences significant at $P \leq 0.01$. ES4, cv. Cultivated; ES5, cv. Highway; ES6, cv. Myway.

Annual garden rocket also achieved a high level of germination when exposed to a range of diurnal temperatures. A small but significant difference was shown for the TGP, with a higher value at the diurnal regime of 24/10°C when compared to 28/16°C (Table 4); however, this difference is likely to be negligible under natural conditions. These results support those of Ellis *et al.* (1993) who found that the germination of annual garden rocket at a diurnal temperature regime of 30/20°C was 95%. A result that is similar to that obtained by the closest diurnal regime used in the present experiment, in which 94% germination was achieved.

Table 4

Effect of diurnal temperatures (12/12 h) on the total germination percentage of annual garden rocket.

Temperature (°C)	TGP
24/10	96.7 a[a]
28/16	93.7 b
18/5	95.4 ab
LSD	2.2

[a]Values in a column followed by the same letter are not significantly different (n = 36), least significant difference 5%. TGP, total germination percentage.

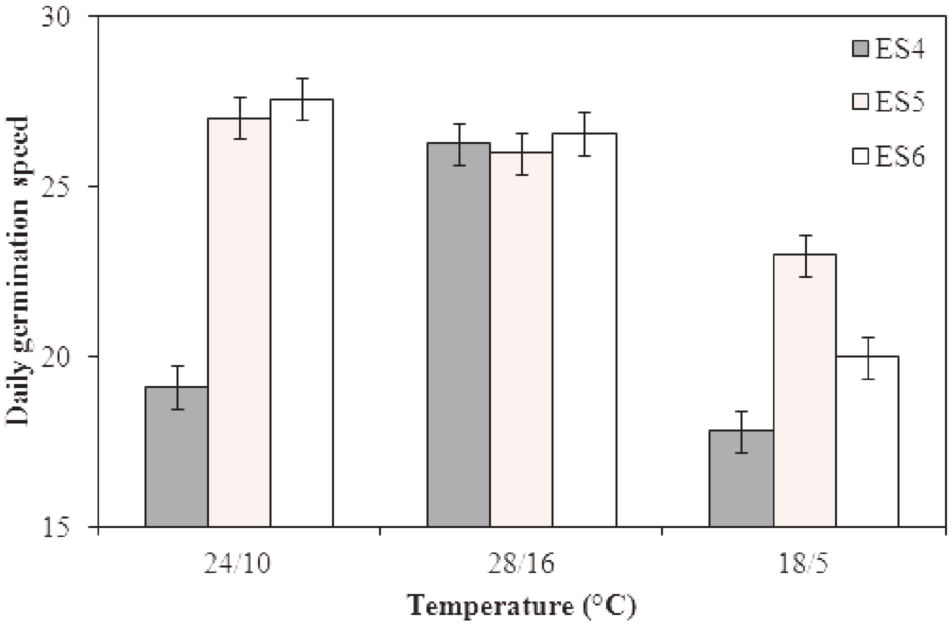

Fig. 8. Interaction effect of cultivar and diurnal temperatures (12/12 h) on the daily germination speed of annual garden rocket; least squares means analysis, n = 12, differences significant at $P \leq 0.01$. ES4, cv. Cultivated; ES5, cv. Highway; ES6, cv. Myway.

There was an interaction between the cultivar and temperature for the DGS and GV of annual garden rocket at the diurnal temperatures (Table 1). At a diurnal regime of 28/16°

C all cvs. had similar DGSs, while higher DGSs were achieved by the cvs. ES5 and ES6 at 24/10°C and the cv. ES5 at 18/5°C (Fig. 8); the results for the GV followed the same trend as the DGS (Fig. 9). These results are similar to those for the constant temperatures, showing that, although this species can achieve comparable TGPs at lower temperatures, the speed at which germination occurs is significantly influenced by the temperature, with higher temperatures resulting in faster germination. This necessitates the requirement of growers to select cultivars with fast germination characteristics .

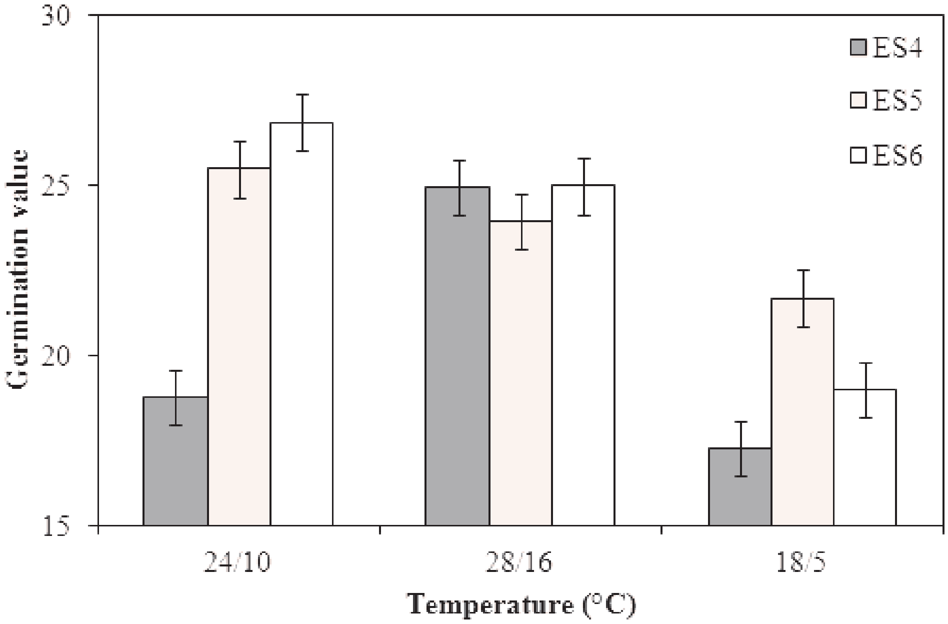

Fig. 9. Interaction effect of cultivar and diurnal temperatures (12/12 h) on the germination value of annual garden rocket; least squares means analysis, n = 12, differences significant at $P \leq 0.01$. ES4, cv. Cultivated; ES5, cv. Highway; ES6, cv. Myway.

CONCLUSIONS

Temperature was more influential in the germination of the smaller perennial seeds than the larger annual seeds; however, the temperature had a significant effect on the speed at which germination occurred in both species. Temperatures between 25 and 30°C resulted in optimal germination for perennial wall rocket, whereas temperatures between 20 and 30°C were optimal for annual garden rocket. These temperatures are similar between the species and reflect the seasonal conditions under which the seeds naturally germinate. Although these species have a similar optimal temperature range for germination, annual garden rocket has a much higher comparative germination rate. The findings of this study have important implications for industry, as the presence of diversity in the commercial cultivars of both rocket species influences the germination of crops, indicating that care must be taken to utilize cultivars with naturally high germination rates.

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