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Effect of soil moisture, soil temperature and seed-borne *Alternaria carthami,* on emergence of safflower (*Carthamus tinctorius* L.)

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Abstract. Clean white seeds of safflower, *Carthamus tinctorius* L. cv. Saffire, from fields in Lethbridge, Alberta, Canada and Portage-la-Prairie, Manitoba, Canada and brown seeds infected with *Alternaria carthami* Chowdhury from Portage-la-Prairie were used to test the effects of soil moisture and temperature on seedling emergence. Seeds were planted in autoclaved soil, at two water potentials (30 kPa and 1500 kPa), and at five temperatures (10, 15, 20, 25, and 30°C) and rated for seedling emergence. The *Alternaria carthami* seeds were 32%, 82%, and 96% infected in the white seeds from Lethbridge, white seeds from Portage-la-Prairie, and brown seeds from Portage-la-Prairies, respectively. For temperatures less than 30°C, emergence of seedlings was greatest (>90%) for the white Lethbridge seed; followed by the white Portage-la-Prairie seed (75–85%); with lowest emergence (50–60%) from the brown Portage-la-Prairie seed, over both soil water potentials. While seed source had significant effects on total seedling emergence, temperature and soil water potential did not. However, post-emergence damping-off increased with higher temperature, and its incidence was greater at high soil moisture (with temperatures above 10°C).

Keywords: *Alternaria carthami*; *Carthamus tinctorius*; Seed-borne; Seedling emergence; Soil water potential; Temperature.

Introduction

Alternaria carthami Chowdhury is an important seedborne pathogen of safflower (*Carthamus tinctorius* L.) in western Canada (Petrie, 1974), U.S.A. (Burns, 1974), Australia (Irwin, 1976), and India (Chowdhury, 1944). Mortensen et al. (1983) found that *A. carthami* was pathogenic on safflower at all growth stages in Montana, causing up to 50% seed rot and seedling blight in susceptible cultivars. In Canada, Howard et al. (1989, 1990) found seedling blight in all fields of safflower surveyed in areas of southern Alberta, with incidence varying from 6 to 53% in 1988 and averaging 52% in 1989. Petrie (1974) found that *A. carthami* occurred in up to 95% of safflower seeds in some fields in Saskatchewan. Also seed-borne inoculum became the primary source of inoculum of leaf spot during the growing season.

Previous investigations have established that safflower seed produced in California and Arizona is generally free of seed-borne pathogens, particularly *A. carthami* (Burns, 1974). Seed produced in Alberta, Manitoba, Saskatchewan, and Montana, however, is often heavily contaminated with this pathogen, resulting in reduced germination and seedling vigour (Mortensen et al., 1983; Mündel, 1996). Fungicidal seed treatments have only partly reduced the incidence of seed-borne *A. carthami*, with the pathogen internal to the seed coat not being killed (Irwin 1976; Mortensen et al., 1983).

Mündel et al. (1995) reported the effects of soil water potential and temperature on damping-off of safflower caused by the soil-borne pathogen *Pythium* sp. "group G", a form of *Pythium ultimum* (Huang et al., 1992). This study was undertaken under a controlled environment to determine the effects of soil moisture and temperature on damping-off and seedling blight of safflower caused by the seed-borne pathogen *A. carthami*.

Materials and Methods

Experiments were performed in temperature-controlled rooms to study safflower seedling emergence and survival, using cv. Saffire (Mündel et al., 1985). Seeds (achenes) of this cultivar are normally white in color. However, infection of seeds by *A. carthami* results in discoloration of the seed coat. Seed samples from three 1991 field variety trials were used: a) white seeds from Lethbridge, Alberta, Canada (WAF); b) hand-selected white seeds from Portage-la-Prairie, Manitoba, Canada (WAC); and c) hand-selected brown seed from the same field in Portage-la-Prairie (BAC). The germination rates of seeds from WAF, WAC, and BAC, rolled in moist paper towelling, kept at ambient room temperature, and counted after four days, were 98%, 84%, and 80%, respectively. Two soil water potentials, low stress (30 kPa) and high stress (1500 kPa), and five temperature regimes (10, 15, 20, 25, and 30°C) were applied to the experimental units.

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Soil Source

Soil (Dark Brown Chernozemic sandy clay loam, Lethbridge series) was gathered from Fairfield Research Farm near Lethbridge, AB, Canada, in 1991, sieved through a 2 mm mesh screen, air dried to constant weight at 20–23°C (5.6% moisture), and stored in large cans at room temperature for use in the experiments. Sterile soil was prepared by autoclaving twice at 121°C for 90 minutes, with 4 days between autoclavings, then covered and left for 4 days before use.

Soil Moisture Adjustment

Sterile distilled water was added to the autoclaved soil to produce the stress levels: (1) 30 kPa (19.3% water vol/vol) and (2) 1500 kPa (12.7% water vol/vol). The water was poured into the bottom of 6 × 6 × 6 cm (L × W × H) plastic containers. Then 150 g autoclaved soil was poured into the containers. The containers were covered with lids and left at room temperature for 1 d to allow uniform distribution of the moisture. The 30 kPa containers were seeded the next day, but the containers with the 1500 kPa treatment were vigorously shaken before seeding to ensure uniform moisture distribution. After seeding, the containers were covered with lids for the duration of the trial. Seedlings were counted without removing the lids, thus maintaining the soil moisture. Moisture loss during the test period was measured for 4 samples per temperature × soil water potential. The 30 kPa containers averaged 11% moisture loss (i.e. compared to the initial moisture %) over the test duration and the 1500 kPa lost 41% of its initial moisture content.

Planting, Incubation and Data Collection

Safflower seeds of WAF, WAC, and BAC were planted 2 cm deep in the soil in each plastic container, with 12 seeds per container, arranged in 3 rows of 4 seeds each, and packed firmly. Containers were covered with lids and placed in rooms at soil temperatures of 10 ± 1, 15 ± 1, 20 ± 1, 25 ± 1, and 30 ± 1°C with a 20 h dark/4 h light cycle. Light (fluorescent and incandescent) was provided to facilitate counting of seedlings and to prevent etiolation. Seedlings were counted as emerged once the cotyledons appeared above the soil surface. Counting for a particular temperature regime was carried out until none of the treatments showed any new emergence for two successive counts.

Some of the emerged seedlings developed dark brown lesions on the cotyledons, which enlarged until the whole seedling was brown and dead; others had dark brown lesions beginning on the hypocotyl and extending up and down until the plant was dead. These seedlings were removed from the containers to eliminate any cross contamination and were recorded as post-emergence damping-off. To confirm the cause of post-emergence damping-off, samples of 100 seedlings showing damping-off symptoms were surface sterilized for 60 seconds in 70% ethanol, air dried on paper towel, and transferred to PDA containing 200 ppm streptomycin in Petri dishes. Cultures were incubated at room temperature (20°C ± 1°C) for 1 week, and the presence of *A. carthami* was then determined.

Experimental Design and Statistical Methods

The seed source (WAF, WAC, or BAC) × soil water potential (30 kPa or 1500 kPa) combinations were randomized in each of three replicate blocks in each of five temperature-controlled rooms (10, 15, 20, 25, and 30°C). Each experimental unit consisted of 5 containers of 12 seeds each. The experiment was carried out three times.

For each experimental unit the days to maximum number of emerged seedlings was recorded and percent emergence calculated. The days to 25% emergence were also determined from logistic regressions as in a previous study on *Pythium* damping-off of safflower (Mündel et al., 1995). Analyses of variance (Snedecor and Cochran, 1980) were carried out over the experimental runs using the General Linear Models (GLM) procedure of the SAS Institute, Inc. (1989). Emergence variables for each temperature and over temperatures were analysed to determine if there were effects on emergence due to seed source, soil water potential, and interactions among these factors. A split-plot model was used with temperatures as main plots and the seed source × soil water potential combinations as subplots. Variance components of whole plots, blocks within whole plots and subplots were estimated. A logit transformation given by $\log_{10}$ [P/(100-P)] (Bartlett, 1947) was applied to the percentage emergence (P) data to stabilize the variance. The emergence and survival response for the period of observation was summarized graphically for each seed source × soil water potential combination at each temperature using a spline plot (SAS Institute, Inc., 1990).

Results

Alternaria carthami was found to be the dominant seedborne pathogen associated with damping-off of safflower. Among the 100-seed samples of BAC seed (brown, Portage-la-Prairie) plated on potato dextrose agar (PDA), *A. carthami* was present in 96% of the seeds, present in 82% of WAC seeds (white, Portage-la-Prairie), and present in just 32% of WAF seeds (white, Lethbridge). In a 100 seed sampling of the most severely infected seed lot (BAC) seedlings showing damping-off symptoms in the controlled-environment study, 78% were infected by *A. carthami*.

The logistic regressions fit the seedling emergence data well (R^2>0.96) and were used to provide an estimate of the days to 25% emergence. Analyses of variance over the temperatures for this variable and percentage emergence indicated significant (P<0.01) interactions of temperature with seed source and soil water potential. Thus, statistical results from analyses of variance (Table 1) and graphs to show the progression of emergence, as well as post-emergence damping-off (Figure 1), are presented separately for each temperature.

Table 1. Effect of soil moisture stress, seed source (Alternaria infection) and temperature on percentage safflower emerged and rate of emergence.

Seed source	Moisture stress (kPa)	Means and SE		
		Emerg. (%)[a]	Days to 25 % emerg.	Days to maximum emerg.
		Temperature = 10°C		
WAF		97.5 a[c]	11.4 b	18.9 a
WAC		77.8 b	11.5 b	20.2 a
BAC		56.9 c	12.1 a	19.2 a
S. E.[b] (df)		0.069 (38)	0.12 (40)	0.69 (38)
	30	84.1 a	11.6 a	19.9 a
	1500	85.8 a	11.8 a	18.9 a
	S.E. (df)	0.056 (38)	0.100 (40)	0.560 (38)
		Temperature = 15°C		
WAF		98.0 a	7.0 b	10.9 a
WAC		76.2 b	7.1 b	11.2 a
BAC		51.6 c	7.5 a	11.6 a
S.E. (df)		0.048 (40)	0.08 (40)	0.34 (40)
	30	85.3 a	7.1 b	11.2 a
	1500	84.2 a	7.3 a	11.3 a
	S.E. (df)	0.039 (40)	0.06 (40)	0.28 (40)
		Temperature = 20°C		
WAF		96.4 a	3.8 b	8.0 a
WAC		79.4 b	3.9 b	6.9 b
BAC		57.1 c	4.1 a	6.8 b
S.E. (df)		0.055 (40)	0.05 (40)	0.30 (40)
	30	85.1 a	3.9 a	7.0 a
	1500	82.3 a	4.0 a	7.5 a
	S.E. (df)	0.045 (40)	0.04 (40)	0.24 (40)
		Temperature = 25°C		
WAF		99.0 a	3.7 a	7.3 a
PW		85.9 b	3.6 b	7.0 a
BAC		60.3 c	3.7 a	7.1 a
S.E. (df)		0.059 (40)	0.03 (40)	0.31 (40)
	30	91.2 a	3.6 a	7.1 a
	1500	89.9 a	3.7 a	7.1 a
	S.E. (df)	0.048 (40)	0.03 (40)	0.26 (40)
		Temperature = 30°C		
WAF		83.5 a	3.9 a	8.4 a
PW		73.0 b	3.8 a	8.1 a
BAC		51.0 c	4.0 a	8.0 a
S.E. (df)		0.071 (27)	0.10 (40)	0.34 (27)
	30	78.2 a	3.5 b	7.9 a
	1500	62.1 b	4.3 a	8.4 a
	S.E. (df)	0.057 (27)	0.08 (40)	0.28 (27)

[a]Emergence (%) means are backtransformed values following a logit transformation.
[b]For emergence (%), standard errors (S.E.) of a nemans are $\log_{10}$ units for comparing transformed neans; the maximum S.E. is presented when the S.E. is not constant for the factor levels.
[c]Means followed by the same letter within a column for a temperature by seed source or soil water potential are not significantly different ($P > 0.05$) according to the least significant difference (LSD) test.

The effect of seed source was very pronounced for percentage emergence at all temperatures, and for days to 25% emergence for 10, 15, and 20°C ($P<0.001$). White seeds from the field in Lethbridge (WAF) achieved higher emergence levels compared to white seeds from the field in Portage-la-Prairie (WAC), which in turn reached higher emergence levels than did brown seeds from the same field in Portage-la-Prairie (BAC) (Table 1). For the lower three temperatures of 10, 15, and 20°C, WAF and WAC reached 25% emergence faster than the BAC seed. The days to maximum emergence were generally similar for the seed sources for the range of temperatures used ($P>0.05$), except for a significant difference ($P<0.05$) at 20°C, with WAF seed requiring 8 days for the larger number of seedlings to emerge compared to near 7 days for the lower numbers emerging with the other two seed sources (Table 1). At 25°C the days to 25% emergence was similar for the seed sources and at 30°C there was no significant differ-

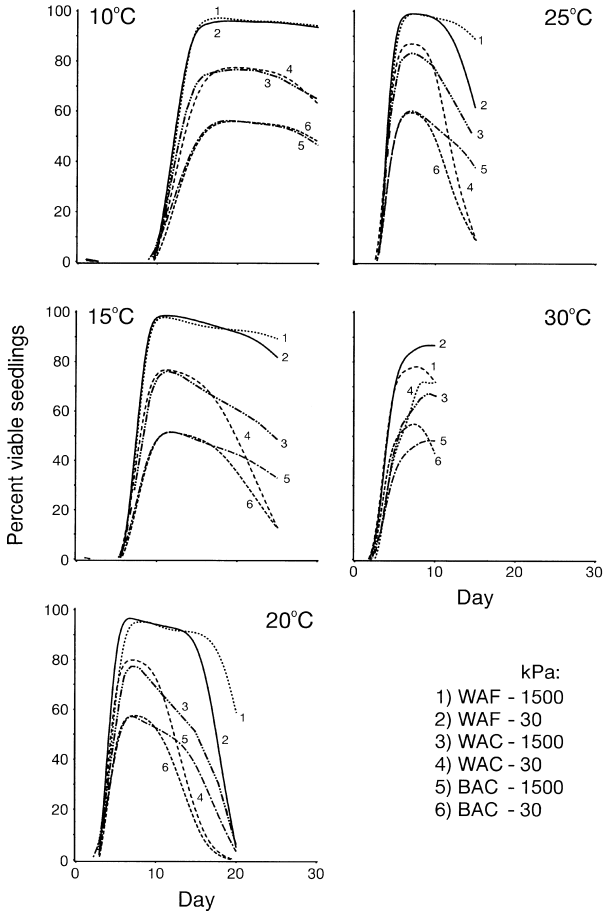

Figure 1. Seedling emergence (%) of Saffire safflower and progression to post-emergence damping-off caused by seedborne *Alternaria carthami* from three seed sources (WAF, WAC, BAC) and two soil water potentials (30 kPa [low stress]; 1500 kPa [high stress]), at 10, 15, 20, 25, and 30° C.

ence in the days to 25% emergence. The percentage emergence was lowered for each of the three seed sources as temperatures increased from 25 to 30°C (Table 1). Temperatures near 15°C commonly occur during emergence in southern Alberta after seeding safflower in the field. Comparing responses of the three seed sources at 15°C, to original germination percentages carried out in moist paper towelling (presented in Materials and Methods, above), seedlings from WAF seed showed no reduction in emergence; WAC seed averaged about a 10% reduction; and BAC seed averaged about a 30% reduction.

The effect of soil water potential on the percentage emergence at temperatures of 10, 15, 20, and 25°C was not significant, and days to 25% emergence were generally similar for both treatments (Table 1). The effect of water potential on percentage emergence and days to 25% emergence at 30° C, however, differed significantly (Table 1). At 30°C, seedlings under the 1500 kPa water potential had a lower percentage emergence and took longer to emerge than did seedlings under the lower 30 kPa moisture stress.

At 20° C, there was a significant ($P<0.05$) seed source × soil water potential interaction for days to maximum emergence. At the low moisture stress level (30 kPa), days to maximum emergence were similar ($P>0.05$) for the seed sources and averaged 7 days. At the high moisture stress level (1500 kPa), the lower total numbers of seedlings emerging with BAC seed took 6.6 (SE=0.42) days to reach maximum emergence, 2.4 days less than the higher number emerging with WAF seed ($P<0.05$).

Post-emergence damping-off was least at 10°C, with neither soil water potential nor seed source affecting it in the period of observation (Figure 1). As temperatures increased, post-emergence damping-off increased and tended to begin earlier. The higher the temperature, the earlier the damping-off began for each seed source, and the faster the damping-off occurred (steeper downward slope). The low moisture stress (30 kPa) generally showed faster damping-off than the high moisture stress treatment, over the temperature range 15, 20, and 25°C. The limited data associated with the short observation period for the 30°C, prevented damping-off observations for the WAF and WAC seed sources, and only initial damping-off observations for the BAC seed.

Discussion

The effect of *Alternaria* seedling blight can be severe over the range of soil moisture and temperatures (10 to 20°C) used in this study. Such environmental conditions are frequently encountered from seeding time to the early part of the growing season in the southern prairies of Canada (Mündel et al., 1995).

The studies carried out indicate that seed-source influences seedling emergence. Seed sources differed with respect to level of infestation with seed-borne *A. carthami*. Seedling damping-off for all three seed sources tested began earlier at higher temperatures and was more severe with an increase in soil moisture. Germination and emergence of safflower seedlings were reduced with increased levels of infection.

The study revealed that the incidence of *Alternaria* damping-off is lower for white colored seeds than for brown-colored seeds. However, visual indicators alone were not sufficient to identify "clean" seed. Borkar and Shinde (1989) determined that up to 84% of apparently healthy seeds of safflower produced various intensities of conidial populations of *A. carthami*. Use of clean and vigorous seed sources to optimize safflower stand establishment is vital. In order to achieve this, seeds should be produced in low humidity environments.

Although visual screening for "white" seed is not adequate to remove the seeds infected by *A. carthami*, sorting by electronic eye (Mündel, 1996) was effective in improving germination percentage from 53% to 75% with two passes through the sorter. Over the temperatures generally encountered at seeding time and for the common range of soil moistures, the level of seed infection with *A. carthami* can be a major factor influencing safflower seed-

ling emergence, where soil-borne pathogens are not a major concern. Joint effects of both the soil-borne pathogens (e.g. *Pythium* spp.) and the seed-borne *Alternaria*, can result in even higher levels of damping-off than those recorded here (unpublished reports by the authors).

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土壤濕度、土壤溫度和種媒菌 *Alternaria carthami* 對紅花（*Carthamus tinctorius* L.）種子出苗率之影響

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本研究測試土壤濕度和土壤溫度對白淨之紅花 (*Carthamus tinctorius* L. cv. Saffire) 種子和受到 *Alternaria carthami* 感染成棕色之紅花種子之幼苗出苗率之影響。紅花種子採自田間，白淨之紅花種子分別採自加拿大 Alberta 之 Lethbridge 和 Manitoba 之 Portage-la-Prairie，而受感染之棕色紅花種子則僅採自 Manitoba 之 Portage-la-Prairie。紅花種子種於高壓滅菌土中，分別以二種土壤濕度 (土壤水勢 30 kPa 和 1500 kPa) 和五種土壤溫度 (10，15，20，25，和 30°C) 處理。來自 Lethbridge 之白淨種子、 Portage-la-Prairie 之白淨種子和 Portage-la-Prairie 之棕色種子受 *Alternaria carthami* 感染琵度分別爲 32%、82% 和 96%。當土壤溫度低於 30°C 時，種子出苗率以來自 Lethbridge 之白淨種子最高 (>90%)，其次是 Portage-la-Prairie 之白淨種子 (75-85%)，而以 Portage-la-Prairie 之棕色種子最低 (50-60%)。土壤濕度之影響不顯著。種子之來源是影響幼苗出苗率之主因，而土壤濕度和土壤溫度之影響則不顯著。然而出苗後之腐敗病因土壤溫度升高而增高，且會因土壤濕度加大而增高 (在土壤溫度高於 10°C 時)。

關鍵詞：*Alternaria carthami* ; *Carthamus tinctorius* ; 種媒 ; 幼苗出苗率 ; 土壤水勢 ; 土壤溫度。