AN ABSTRACT OF THE THESIS OF

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Title: THE ROLE OF TEMPERATURE IN DETERMINING THE INHIBITORY ACTIVITIES OF AMBROSIA ARTEMISIIFOLIA, AMBROSIA TRIFIDA, BROMUS INERMIS AND HELIANTHUS ANNUS ON SPRING AND WINTER WHEAT (TRITICUM AESTIUUM)

Abstract approved: John Parrish, Robert L. Parenti
Co-Chairman

An investigation was conducted to determine the effects of varying temperatures between 20 to 30 C day and 10 to 15 C night on the inhibitory activities of aqueous extracts of Ambrosia artemisiifolia, Ambrosia trifida, Bromus inermis and Helianthus annus on Spring and Winter wheat germination and growth.

The results showed that Winter wheat seed germination was significantly less in the presence of extracts at 24/12, 28/14 and 30/15 than under a 20/10 C (day/night) temperature regime, whereas Spring wheat showed no dramatic extract inhibition under any of the

temperature regimes. This might be due to genetic differences between Spring and Winter wheat and, thus, allowing Spring wheat to be competitive with other Spring weedy species.

The dramatic significant reduction in dry weight of both Spring and Winter wheat seedlings grown for ten days in the aqueous extracts is considered to be of tremendous economic importance to wheat agriculturists. The striking reductions in productivity (10 - 65 percent) caused by the extracts would seriously reduce wheat yield if those inhibitory species are permitted to grow on the same field as wheat.

Although the exact nature of the alleopathic agents in the plants tested in this study is not known, it is apparent that further research is necessary in order to isolate the specific inhibitory compounds. Once isolated, further investigations can be pursued that will allow elucidation of the mechanism(s) involved in alleopathy. Isolation of the alleopathic agents might also suggest a possible explanation as to why those agents demonstrated greater inhibitory activities on wheat germination and growth under temperature regimes above.

THE ROLE OF TEMPERATURE IN DETERMINING THE INHIBITORY ACTIVITIES OF *AMBROSIA ARTEMISIIFOLIA*, *AMBROSIA TRIFIDA*, *BROMUS INERMIS* AND *HELIANTHUS ANNUS* ON SPRING AND WINTER WHEAT (*TRITICUN ABSTIUUM*)

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by

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D. E.

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INTRODUCTION

It has been generally accepted, until recently, that competition between higher plants is mainly dependent on the relative abilities of the plants to obtain the necessary nutrients, water and light required for their growth and reproduction. And it has been documented that the production of wastes and by-products, many of them toxic to some degree, is a normal consequence of life processes. It is to be expected, therefore, that in some circumstances these chemicals, especially when excreted by plants, will accumulate in the environment to levels having significant inhibitory effects on other plant species or even on the plants themselves. Agrostemma githago (garland), for instance, cannot germinate in fields of Beta vulgaris (sugar beet), because sugar beet excretes a substance which inhibits the germination of garland (Evanori, 1949). This kind of effect shows a similar natural function of antibiotic activity excreted by soil fungi or bacteria which inhibit or destroy other soil organisms in the same locality.

Numerous studies of similar observations suggest that many plants have chemicals which inhibit the growth and distribution of other species. Parenti and Rice (1969) showed that substances were produced by Digitaria sanguinalis, an early invader in old field succession, which inhibited not only the germination and growth of associated species but also inhibited the growth and development of its own seedlings, thereby serving as a built-in population control mechanism. Jackson and Willemson (1976) found that Ambrosia

artemisiifolia (common ragweed) and Raphanus ranphanistrum (wild radish), the early invaders in the first year of old field succession, failed to become re-established in plots cleared of second stage vegetation dominated by Aster pilosus, despite the large number of seeds of these primary invaders present in the soil. Willemsen and Rice (1972) found phenolic acids in ragweed seeds and suggested that some may act as germination inhibitors. Wilson and Rice (1968) found isochlorogenic and chlorogenic acid to be the allelopathic agents (a chemical compound which inhibits the growth of another plant in the same habitat) in Helianthus annus. Rice (1964, 1965) found that many of the low-nitrogen requiring pioneer species of seed plants produced inhibitory substances which gave them a selective advantage in competition over plants with higher nitrogen requirements. O'Donnell (1972) found that aqueous extracts of Cannabis sativa (hemp) greatly inhibited the germination of Bromus japonicus, Helianthus annus, and Setaria viridis. These germination inhibitors are very interesting because they seem to fulfill a biological function and must be considered just as important in the study of life cycles of plants as growth hormones and other stimulating substances.

Although much information has been accumulated on the inhibitory activities of some species of plants, the role of temperature, especially the effect of varying temperature with the physiological problems associated with it, still remains to be investigated. Tendler, Korman and Nishmota (1967) found that at 30 to 50 C Eumycophyta did not produce the enzyme caseinase but did so at lower temperatures. Borek and Walesh (1951) showed that a temperature

difference of only 2 C determines whether lactobaccillus can produce phenylalaline. The temperature at which phenylalaline occurred was at 35 C, but below that temperature range it did not. Murull (1971) observed that a temperature regime of 32 day and 27 C night decreased mineral uptake in wheat plants compared to a lower temperature schedule of 21 day and 15 C night. Paulsen and Rotini (1968) observed too that soybeans grown under growth chamber conditions in a 30/20 C day and night sequence contained lower concentrations of phosphorus than those at 20/10 C day and night temperatures. Therefore, the objective of this study was to determine the effects of varying temperatures on the inhibitory activities of Ambrosia artemisiifolia, Ambrosia trifida, Bromus inermis and Helianthus annus on two varieties of wheat, commonly grown in the United States and which are also being introduced in the Savannah regions of Nigeria, which have warm temperatures (high temperature by day and low temperature by night) and abundant rainfall.

MATERIALS AND METHODS

Ambrosia artemisiifolia (common ragweed), Ambrosia trifida (giant ragweed), Bromus inermis (brome grass) and Helianthus annus (sunflower) were collected at Ross Natural History Reservation, which is located about fourteen miles northwest of Emporia. Winter wheat seeds were obtained from a local seed dealer in Topeka, Kansas, while Spring wheat seeds were obtained from Younky Farms in Wayne, Nebraska.

Extracts of each test plant collected from the wild were made by grinding 10 g fresh weight of plant material (leaves and shoot) with 100 ml of distilled water in a Waring Blendor for ten minutes. After allowing the mixtures to stand for five minutes they were filtered through cheese cloth in a Buchner funnel. Two hundred seeds of each variety of wheat were placed in Petri dishes, fifty seeds per Petri dish, containing filter paper disks saturated with 5 ml of each extract solution. These were placed in growth chambers (Percival, Model PT 80) with an average illumination intensity of 1200 foot-candles and a 12:12 hour (light:dark) cycle. The experiments were conducted in triplicate with the following day/night temperature regimes: 20/10, 24/12, 28/14 and 30/15 C. The control groups were kept at the same temperatures as the test seeds but wetted with distilled water only.

Germination counts were made after twenty-four hours and subsequently every twelve hours for six days. In testing seedling growth and development, three replicates of 100 seeds each of winter and spring wheat were germinated in quartz sand and allowed to grow

for fourteen days. The growing seedlings were supplied with a complete nutrient solution (Hoagland and Aron, 1950). After the fourteen days the seedlings were transferred to plastic vials (4 ml volume) containing a 1:5 ratio of nutrient solution to plant extract and allowed to grow for ten days in a growth chamber. The controls were grown in a 1:5 ratio of nutrient solution to distilled water. The nutrient and extract solutions were changed after five days. On the tenth day, the seedlings were harvested, dried for forty-eight hours at 37 C and the oven dry weight determined to the nearest 0.01 g.

Statistical Treatments

All 48 hour and 144 hour germination counts were subjected to square root transformations in order to meet requirements for normality of distribution. Following transformation, the data were statistically analyzed by two-way analysis of variance with replication (Sokal and Rohlf, 1969). Individual statistical differences were ascertained by use of Duncan's Multiple Range Test (Steel and Torrie, 1960). Some data were analyzed by Student's Paired "t" test. All data were considered significant if they equaled or exceeded table "F" or "t" values at the 0.05 level of probability.

RESULTS

The four tables show the number of seeds that germinated under the various temperature regimes at each measured time interval. The last column of each table shows the mean ± S.E. of the total percentage of the seeds that germinated at the end of the 144 hour period.

Effects of Extracts Under a 20/10 C, Day/Night, Regime

Common ragweed, giant ragweed, brome grass and sunflower extracts greatly reduced germination for the first 24 hour period (Table 1). Giant ragweed extract was the most potent inhibitor since only less than 2 percent of both Spring and Winter wheat germinated during the first 24 hour period.

Germination was completed in the control group at the end of the 72 hour period. In the test groups of Spring and Winter wheat seeds it took 108 hours to complete germination of those seeds treated with common ragweed, brome grass and sunflower extracts. It took 96 hours to complete the seed germination of those seeds treated with giant ragweed extract.

Less than 35 percent of both wheat seeds treated with ragweed, brome grass and sunflower extracts grew radicles. The first plumule was observed in Spring wheat seeds treated with brome grass, sunflower and ragweed extracts after 72 hours, while in all extract-exposed Winter wheat groups, plumules were not observed until after 84 hours. Plumules were first observed after 48 hours in both control groups.

Table 1. The Effects of Aqueous Extracts of *Ambrosia artemisiifolia*, *Ambrosia trifida*, *Bromus inermis* and *Helianthus annus* on the Number of Spring and Winter Wheat Seeds Germinated Under a 20/10 C, Day/Night, Temperature Regime.

Extract		Time (Hours)												
		24	36	48	60	72	84	96	180	120	132	144	Total % Germination	±SE
Ambrosia artemisiifolia	a[1]	5	91	162	170	176	179	180	181	181	181	181	90.5	±0.44
	b[2]	20	152	158	171	184	186	188	189	189	189	189	94.5	±0.01
Ambrosia trifida	a	3	87	113	154	171	176	178	178	178	178	178	89.0	±4.42
	b	4	90	153	173	187	188	189	191	191	191	191	99.5	±1.15
Bromus inermis	a	33	145	168	175	182	185	188	189	189	189	189	94.5	±1.73
	b	21	149	157	186	189	190	191	192	193	193	193	96.5	±0.11
Helianthus annus	a	13	62	125	167	177	179	179	179	179	179	179	89.5	±4.04
	b	27	151	173	181	188	191	192	193	193	193	193	96.5	±0.91
Control	a	54	163	187	192	196	196	196	196	196	196	196	98.0	±0.01
	b	73	163	182	194	196	196	196	196	196	196	196	98.0	±0.01

[1] a = Spring wheat

[2] b = Winter wheat

Statistical analysis at the 48 hour period showed that extract-exposed Spring wheat seeds were significantly inhibited by both temperature and extract, although no temperature-extract interactions were found to be present (Figure 1). Spring wheat germination was significantly less than the controls only in the presence of giant ragweed extract. Winter wheat germination at 48 hours was significantly less than the controls in all of the extracts except sunflower extract exposed to the 20/10 C regime (Figure 2). At 144 hours, Spring wheat and Winter wheat germination were not significantly inhibited by any of the aqueous extracts under the 20/10 C temperature regime (Figures 3 and 4).

Effect of Extracts at 24/12 C, Day/Night, Regime

There were dramatic reductions in the percent germination of both Spring and Winter wheat treated with all of the extracts at the end of 24 hours (Table II). By the end of the first 24 hour period, Spring wheat treated with common ragweed extract showed 14 percent, giant ragweed 2 percent, brome grass 19.5 percent and sunflower 7.5 percent. In contrast, the germination for Winter wheat in common ragweed extract was 10.5 percent, giant ragweed 9 percent, brome grass 37.5 percent and sunflower 19.5 percent. The percent germination for the control groups were 60.5 percent for Spring wheat and 63 percent for Winter wheat at the same period. Both Spring and Winter wheat treated with common ragweed extract showed the same percent germination at the end of the 60 hour period. Germination was completed early (84 hours) in the control group for Spring and Winter

Figure 1. The effects of aqueous extracts of Ambrosia artemisiifolia, Ambrosia trifida, Bromus inermis and Helianthus annus on the number (square root transformation) of Spring wheat seeds germinated after 48 hours, under 20/10, 24/12, 28/14 and 30/15 C (day/night) temperature regimes. Those data points with the same symbols show no significant differences in the effects of temperature (a or b) or extract (w or x) on germination.

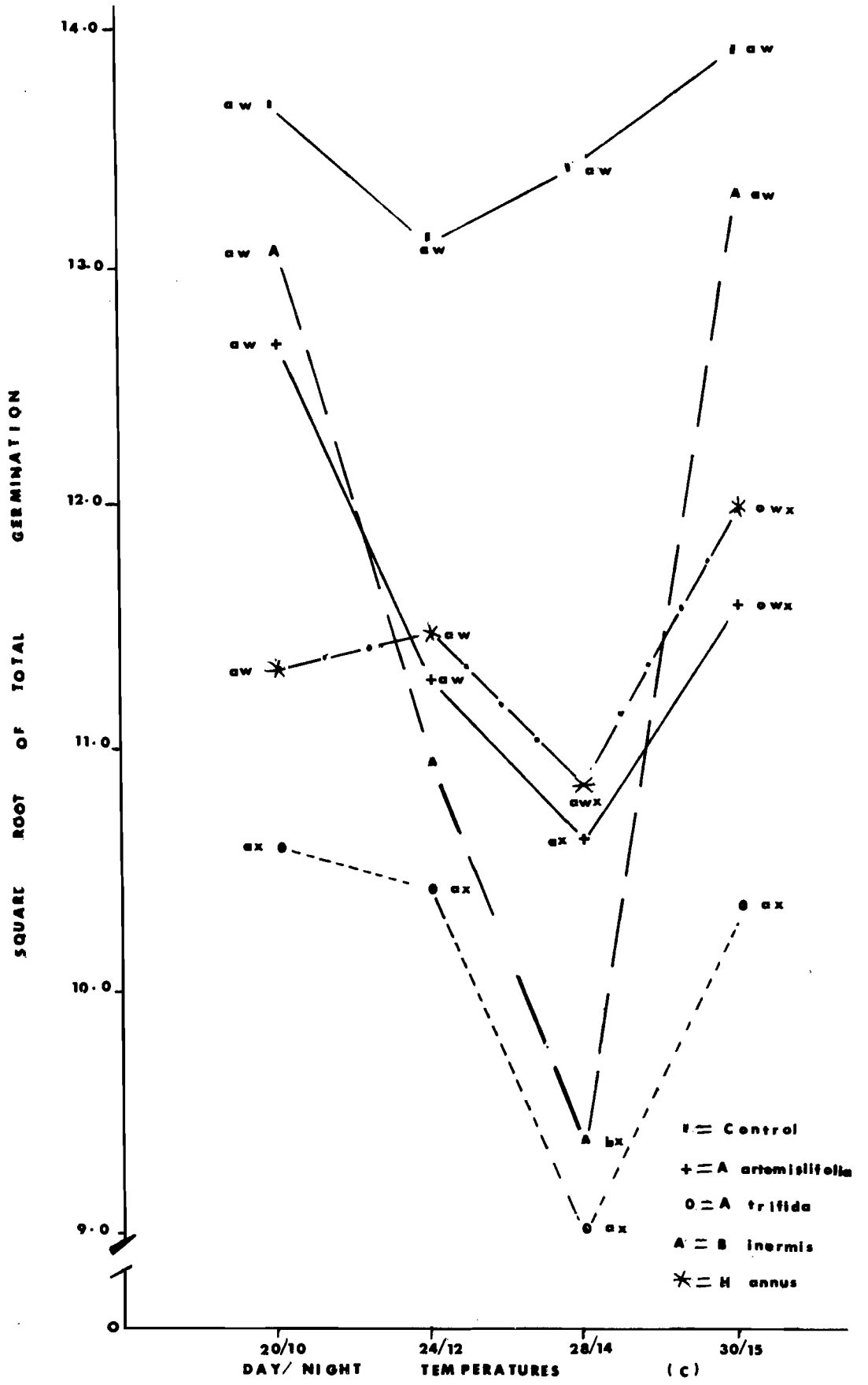
SQUARE ROOT OF TOTAL GERMINATION
14·0
13·0
12·0
11·0
10·0
9·0
0
20/10
24/12
28/14
30/15
DAY/ NIGHT TEMPERATURES (C)
I = Control
+ = A artemisiifolia
0 = A trifida
A = B inermis
* = H annus

Figure 2. The effects of aqueous extracts of Ambrosia artemisiifolia, Ambrosia trifida, Bromus inermis and Helianthus annus on the number (square root transformation) of Winter wheat seeds germinated after 48 hours, under 20/10, 24/12, 28/14 and 30/15 C (day/night) temperature regimes. Those data points with the same symbols show no significant differences in the effects of temperature (a, b, or c) or extracts (w, x or y) on germination.

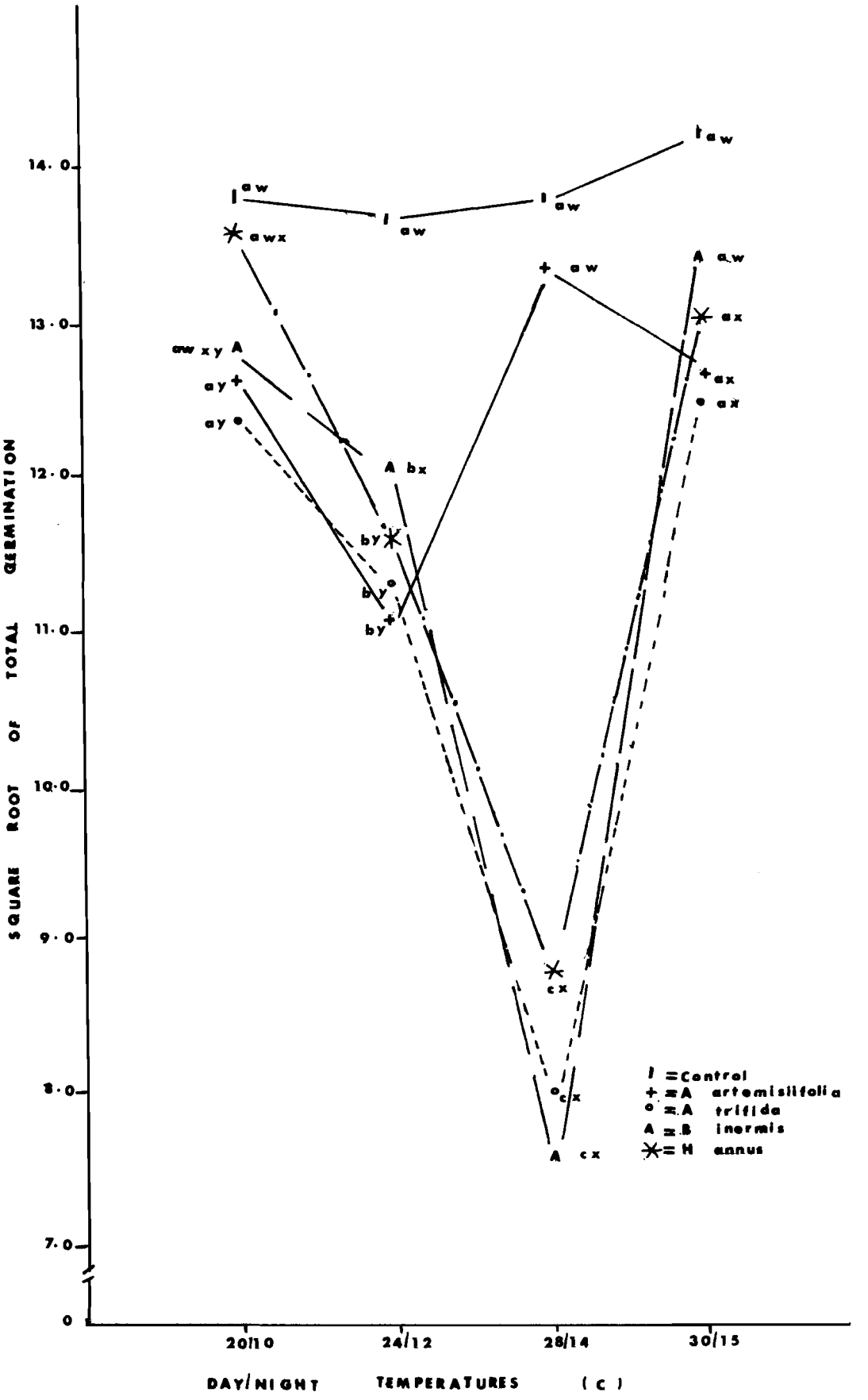
SQUARE ROOT OF TOTAL GERMINATION
14·0
13·0
12·0
11·0
10·0
9·0
8·0
7·0
0
20/10
24/12
28/14
30/15
DAY/NIGHT TEMPERATURES (C)
I =Control
+ =A artemisiifolia
° =A trifida
A =B inermis
✳ = H annus

Figure 3. The effects of aqueous extracts of *Ambrosia artemisiifolia*, *Ambrosia trifida*, *Bromus inermis* and *Helianthus annus* on the number (square root transformation) of Spring wheat seeds germinated after 144 hours, under 20/10, 24/12, 28/14 and 30/15 C (day/night) temperature regimes. Those data points with the same symbols show no significant differences in the effects of temperature (a or b) or extracts (w or x) on germination.

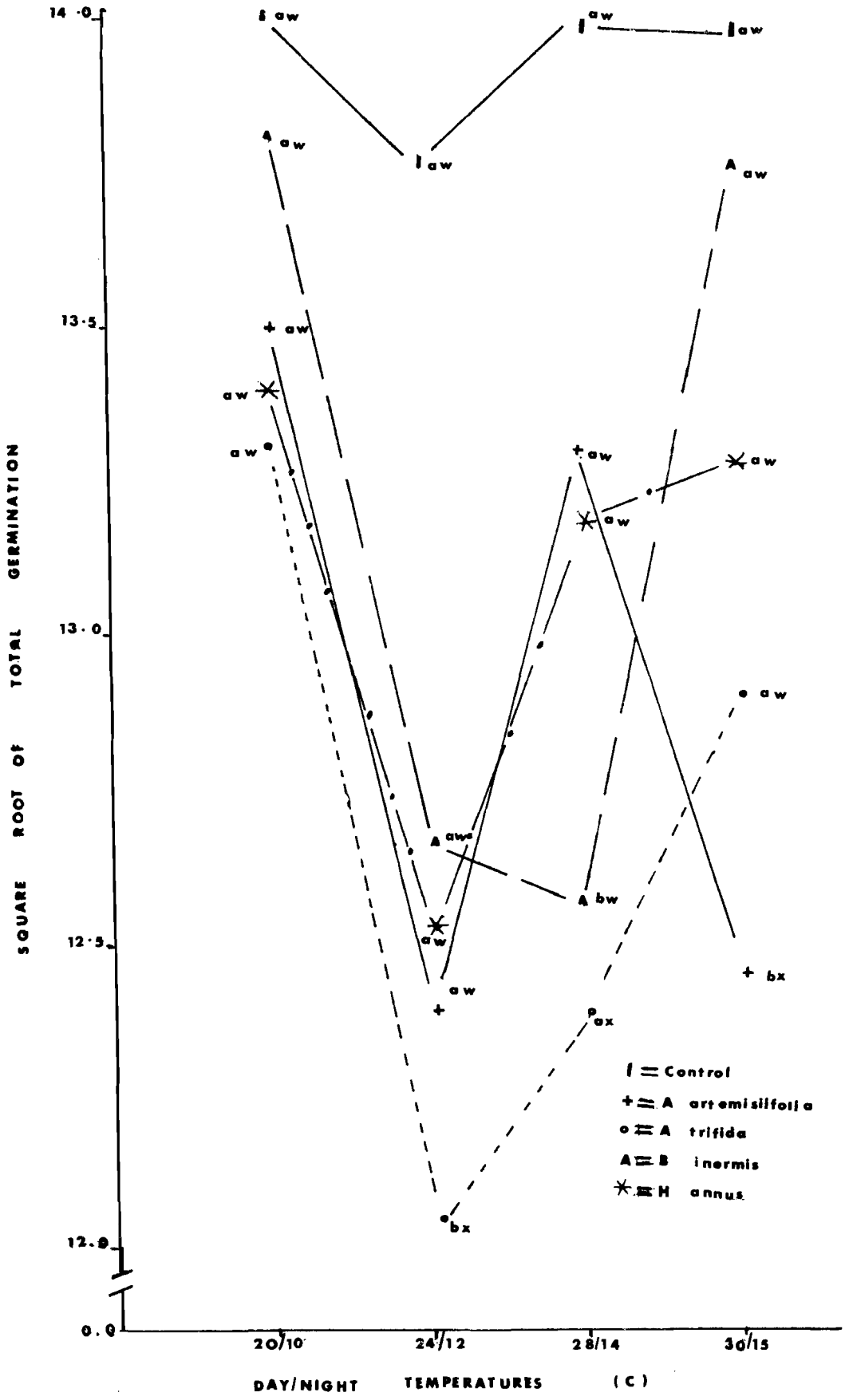
14·0
13·5
13·0
12·5
12·0
0.0
SQUARE ROOT OF TOTAL GERMINATION
20/10
24/12
28/14
30/15
DAY/NIGHT TEMPERATURES (C)
I = Control
+ = A artemisiifolia
o = A trifida
A = B inermis
✳ = H annus

Figure 4. The effects of aqueous extracts of Ambrosia artemisiifolia, Ambrosia trifida, Bromus inermis and Helianthus annus on the number (square root transformation) of Winter wheat seeds germinated at 144 hours, under 20/10, 24/12, 28/14 and 30/15 (day/night) temperature regimes. Those data points with the same symbols show no significant differences in the effects of temperature (a, b or c) or extracts (w, x, y or z) on germination.

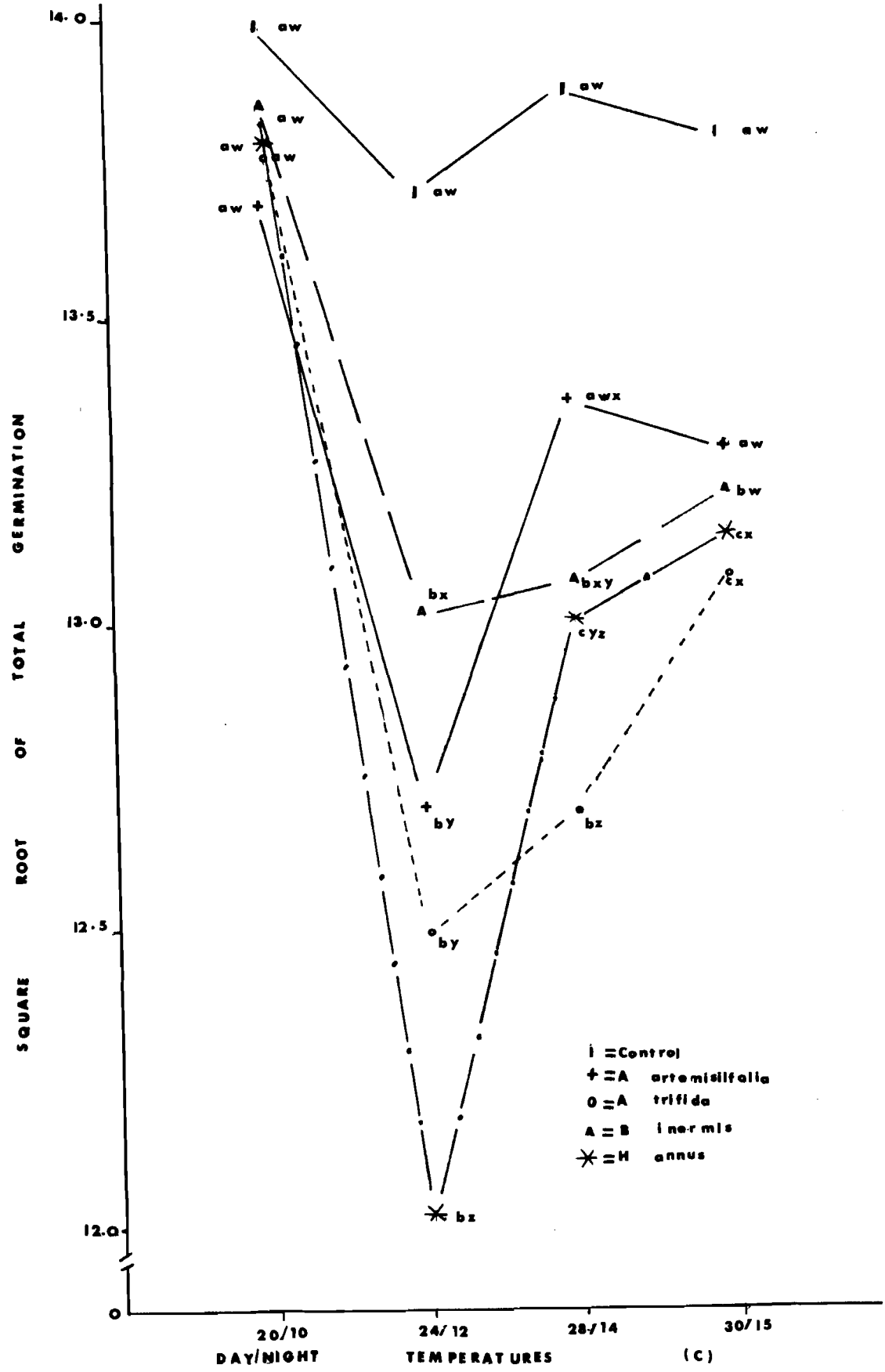
14·0
13·5
13·0
12·5
12·0
0
SQUARE ROOT OF TOTAL GERMINATION
20/10
24/12
28/14
30/15
DAY/NIGHT TEMPERATURES (C)
i =Control
+ =A artemisiifolia
0 =A trifida
A = B inermis
✳ =H annus

Table II. The Effects of Aqueous Extracts of *Ambrosia artemisiifolia*, *Ambrosia trifida*, *Bromus inermis* and *Helianthus annus* on the Number of Spring and Winter Wheat Seeds Germinated Under a 24/12 C, Day/Night, Temperature Regime.

Extract		Time (Hours)												
		24	36	48	60	72	84	96	108	120	132	144	Total % Germination	±SE
Ambrosia artemisiifolia	a[1]	28	97	124	136	144	149	151	154	155	155	155	77.5	±2.94
	b[2]	21	83	119	136	146	150	154	157	159	159	159	79.5	±0.39
Ambrosia trifida	a	4	60	108	126	133	137	140	143	144	144	144	72.0	±0.57
	b	18	87	121	134	142	148	151	154	155	155	155	77.5	±1.73
Bromus inermis	a	39	96	133	146	151	154	157	158	158	158	158	79.0	±0.57
	b	75	108	142	155	165	169	169	169	169	169	169	84.5	±4.6
Helianthus annus	a	15	80	131	144	148	151	152	154	155	155	155	77.5	±0.70
	b	39	98	123	133	137	143	144	144	144	144	144	72.0	±1.69
Control	a	121	169	181	184	185	185	185	186	186	186	186	93.0	±0.01
	b	126	162	178	184	185	187	187	187	187	187	187	93.5	±0.01

[1]a = Spring wheat

[2]b = Winter wheat

wheat compared to the test seeds. In both ragweed and brome grass extracts germination was completed at 108 hours for the Spring wheat; for Winter wheat it was 84 hours in brome grass extract and 120 hours in sunflower.

Statistical analysis revealed that at 48 hours both temperature and extracts significantly affected Spring and Winter wheat germination (Figures 1 and 2). In the presence of giant ragweed, Spring wheat was significantly reduced at 24/12 C. Temperature-extract interactions did not occur in Spring wheat but were significant in Winter wheat where germination was significantly inhibited by temperature-extract interactions in the presence of giant ragweed, common ragweed, brome grass and sunflower extracts.

By the end of 144 hours, there were significant effects of the extracts on both Spring and Winter wheat. Common ragweed, giant ragweed and brome grass significantly reduced Spring wheat total germination at 24/12 C (Figure 3). Winter wheat total germination was significantly inhibited by temperature-extract interactions in the presence of all extracts at 24/12 C (Figure 4). The germination of Winter wheat in the presence of sunflower extract was significantly lower under the 24/12 C temperature regime than under all the other temperature regimes.

Effect of Extracts Under a 28/14 C, Day/Night, Regime

Spring wheat seeds treated with common ragweed showed the same percent germination, 8 percent, as those treated with giant ragweed by the end of the first 24 hour period (Table III). Brome grass

Table III. The Effects of Aqueous Extracts of *Ambrosia artemisiifolia*, *Ambrosia trifida*, *Bromus inermis* and *Helianthus annus* on the Number of Spring and Winter Wheat Seeds Germinated Under a 28/14 C, Day/Night, Temperature Regime.

Extract		Time (Hours)												
		24	36	48	60	72	84	96	108	120	132	144	Total % Germination	±SE
Ambrosia artemisiifolia	a[1]	16	63	106	137	158	169	172	172	173	173	173	86.5	±1.24
	b[2]	65	120	168	176	178	180	181	181	181	181	181	90.5	±1.24
Ambrosia trifida	a	16	42	82	117	128	136	142	148	154	154	154	77.0	±1.36
	b	28	43	62	148	156	158	159	160	160	160	160	80.0	±3.09
Bromus inermis	a	17	67	86	101	118	135	147	153	156	156	156	78.0	±1.41
	b	10	45	60	155	162	168	169	170	171	171	171	85.5	±0.46
Helianthus annus	a	15	41	115	159	164	174	177	179	179	179	179	89.5	±0.32
	b	21	65	73	152	165	167	169	169	169	169	169	84.5	±1.26
Control	a	35	148	177	194	195	195	195	195	195	195	195	97.5	±0.01
	b	59	148	178	184	189	190	191	191	191	191	191	95.5	±0.01

[1]a = Spring wheat

[2]b = Winter wheat

showed 8.5 percent and sunflower 7.5 percent of the controls at the same period (Table III). Winter wheat treated with common ragweed showed 32.5 percent, giant ragweed 14 percent, brome grass 5 percent and sunflower 10.5 percent of the controls by the end of the first 24 hour period (Table III). First plumule and radicles were observed in Spring wheat after the 60 hour period and after 84 hours in Winter wheat. Germination in the control groups was completed after the 72 hour period for both Spring and Winter wheat. In contrast, germination was not completed for Spring wheat in common ragweed extract until the end of 108 hours, while it was 96 hours for Winter wheat; in giant ragweed it was 120 hours for Spring wheat and 108 hours for Winter wheat, while in brome grass it was 120 hours for both.

At the 48 hour period both of the ragweeds and brome grass extracts significantly reduced Spring wheat germination (Figure 1). In addition Spring wheat germination in the presence of the giant ragweed extract was significantly lower at 28/14 than at 20/10, 24/12 or 30/15 C. Winter wheat was significantly inhibited by both temperature and extracts in the presence of giant ragweed, brome grass and sunflower extracts, but by neither temperature nor extract exposure in common ragweed extract during the first 48 hour period (Figure 2).

Statistical analysis at the 144 hour period showed that Spring wheat was significantly inhibited by giant ragweed, but not by common ragweed, brome grass and sunflower extracts (Figure 3). Temperature significantly inhibited germination of Spring wheat only in the presence of brome grass. At the 144 hour period, temperature-extract interactions significantly inhibited Winter wheat germination in the

presence of giant ragweed, brome grass and sunflower extracts (Figure 4). Percent germination of Winter wheat exposed to giant ragweed extract was lower than those exposed to extracts of brome grass, but not different from sunflower extract-treated seeds.

Effect of Extracts Under a 30/15 C, Day/Night, Regime

The aqueous extracts of all the test plants caused dramatic decreases in percent germination for both Spring and Winter wheat compared to the controls at 24 hours (Table IV). Spring wheat treated with sunflower extract showed the greatest inhibition since only one percent of the seeds germinated by the end of the first 24 hours period. Germination was 17.5 percent in common ragweed, 2 percent in giant ragweed and 3 percent in the brome grass for the first 24 hour period. In contrast, germination of Winter wheat was 11 percent and 27 percent of the controls in the presence of common and giant ragweed extracts, respectively.

At the end of 48 hours, only the giant ragweed extract significantly inhibited Spring wheat at 30/15 C (Figure 1). However, giant and common ragweed and sunflower extracts showed significant inhibition of germination of Winter wheat at the 48 hour period (Figure 2).

Statistical analysis at the end of the 144 hour period also showed that there were significant effects on both Spring and Winter wheat. Spring wheat germination, however, only was significantly inhibited in the presence of the giant ragweed extract (Figure 3). Winter wheat germination was significantly inhibited, in contrast,

Table IV. The Effects of Aqueous Extracts of *Ambrosia artemisiifolia*, *Ambrosia trifida*, *Bromus inermis* and *Helianthus annus* on the Number of Spring and Winter Wheat Seeds Germinated Under a 30/15 C, Day/Night, Temperature Regime.

Extract		Time (Hours)												
		24	36	48	60	72	84	96	108	120	132	144	Total % Germination	±SE
Ambrosia artemisiifolia	a[1]	35	120	133	142	149	151	153	154	154	154	154	77.0	±0.47
	b[2]	54	141	157	168	175	179	179	179	179	179	179	89.5	±2.63
Ambrosia trifida	a	4	50	109	126	148	165	166	167	167	167	167	83.5	±2.33
	b	22	64	156	168	172	173	173	173	173	173	173	86.5	±0.81
Bromus inermis	a	6	168	178	183	186	189	189	190	190	190	190	95.0	±0.47
	b	78	164	173	174	176	178	178	178	178	178	178	89.0	±0.19
Helianthus annus	a	2	121	142	154	169	171	173	174	174	174	174	87.0	±0.19
	b	9	112	157	169	172	174	174	174	174	174	174	87.0	±0.74
Control	a	121	178	191	194	194	194	194	195	195	195	195	97.5	±0.01
	b	139	179	186	189	190	190	190	190	190	190	190	95.0	±0.01

[1]a = Spring wheat

[2]b = Winter wheat

by a temperature-extract interaction in the presence of both giant ragweed and sunflower extracts, but not by common ragweed or brome grass extracts (Figure 4). The 30/15 C temperature regime also had a significant effect on total germination of brome grass-exposed Winter wheat.

Effects of Aqueous Extracts on Wheat Seedlings at 20 C

Giant ragweed, brome grass and sunflower extracts appreciably reduced the weights of both wheat seedlings. The Winter wheat seedlings grown in common ragweed extract for two weeks showed the greatest inhibitory effect since those seedlings produced significantly less dry matter, 1.95 gm less, than the controls (Table V). Winter wheat grown in giant ragweed extract showed 0.82 gm less, in brome grass 0.59 gm less, and in sunflower 0.18 gm less dry weight than the controls grown in complete nutrient solution.

Spring wheat exhibited a 1.07 gm difference for those seeds grown in brome grass extract compared to the controls. In common ragweed extract the difference was 0.34 gm, while in giant ragweed it was 0.38 gm and in sunflower it was 0.61 gm lower than the controls (Table V). Chlorosis was observed in the leaves of more than 50 percent of the extract-exposed seedlings, especially along the veins.

Statistical analysis showed that only the common ragweed extract failed to significantly reduce the dry weight of Winter wheat seedlings, whereas all of the extracts significantly inhibited Spring wheat seedling growth, even at the 0.001 level of significance (Table V).

Table V. The Effects of Aqueous Extracts on the Growth and Development of Three Replicates of 100 Wheat Seedlings at 20 C.

Extract		Mean ± SE Oven-dry weight (g)	% of Control
Control	a[1]	3.02 ± 0.01	--
	b[2]	3.62 ± 0.02	--
Ambrosia artemisiifolia	a	1.08 ± 0.01	35.6***[3]
	b	3.28 ± 0.33	90.6
Ambrosia trifida	a	2.01 ± 0.01	66.6***
	b	2.24 ± 0.37	61.9*
Bromus inermis	a	2.44 ± 0.02	80.5***
	b	2.55 ± 0.19	70.4*
Helianthus annus	a	2.85 ± 0.04	94.1*
	b	3.01 ± 0.06	83.1**

[1]a = Spring wheat

[2]b = Winter wheat

[3]Statistically different from respective control values at the following levels of probability: * = 0.05, ** = 0.01, and *** = 0.001.

DISCUSSION

Early Inhibitory Effects of Extracts

The experimental results showed that during the first 24 hour period both Spring and Winter wheat seed germination and growth were strikingly inhibited by Ambrosia artemisiifolia, Ambrosia trifida, Bromus inermis and Helianthus annus extracts. The lowest percent of germinated seeds occurred in extract-exposed wheat seeds at the 24/12 C and 30/15 C regimes, but all extracts showed remarkable inhibition of both wheat varieties at all temperatures, except Spring wheat in the presence of common ragweed extract at 28/14 C. The percent germination was highest at all temperatures in the control groups which were not exposed to the extracts.

At the end of the 48 hour period there were no significant effects of temperature on the control groups, although germination was slightly lower at 20/10 and 28/14 C than at the 24/12 and 30/15 C temperature regimes. There also were no significant effects of temperature on extract-exposed Spring wheat at 20/10, 24/12 and 30/15 C, however, Spring wheat germination in the presence of brome grass extract was significantly inhibited by the 28/14 C temperature regime, where all of the extracts produced their greatest inhibitory effects (Figures 1 and 2). Spring wheat is known to withstand greater concentrations of chemical compound when in competition with weedy plants for germination, growth and development of seedlings (Parenti, 1977). Winter wheat exposed to Bromus inermis, Ambrosia trifida and

Helianthus annus, likewise, was inhibited to the greatest extent at 28/14 C while Ambrosia artemisiifolia demonstrated its greatest inhibitory activity at 24/12 C regime. These results seemed to suggest that 24 hours was not a long enough time to reveal the accurate germination pattern for both Spring and Winter wheat. The 48 hour germination patterns were considered to be more indicative of germination patterns since statistical analysis showed many significant extract effects during that period. The 48 hour period also is considered important because the germination of plants is more competitive within the first 48 hours. The seeds would be expected to be at a point of high mitotic division and cell elongation, and hence, very susceptible to inhibition. It has been shown (Wilson, 1970) that inhibition may occur due to, at least in part, a suppression of both cell elongation and cell division. Under these circumstances, inhibition probably occurred soon (within 48 hours) after the wheat seeds were planted.

Late Inhibitory Effects of Extracts

Germination patterns of both Spring and Winter wheat seeds at the later periods were in many cases much different from early patterns. Several instances indicated a recovery from early extract inhibitory activities. This was best illustrated by the strong inhibition of Spring wheat by sunflower extract at 48 hours and the reduced effect of that extract at 144 hours under the 24/12 C temperature regime. Several examples of the opposite situation are best shown in the case of common ragweed's effect on Winter wheat

under the 30/15 C temperature regime. In that case, early inhibition at 48 hours was minimal, however, at 144 hours common ragweed extract was the most potent inhibitor of total Winter wheat germination (Figures 3 and 4).

Germination in the control groups under 24/12 C was completed at the end of the 72 hour period in both Spring and Winter wheat and all of those that germinated bore root hairs. Both Spring and Winter wheat exposed to giant ragweed and sunflower had the least percent germination under the 24/12 C regime, indicating that giant ragweed and sunflower extracts may contain high concentrations of inhibiting substances. Lack of root hairs in the seeds that germinated in the presence of giant ragweed and sunflower extracts add to the speculation that these two plant species were exceptionally potent inhibitors of wheat germination.

Percent germinations for Spring wheat were lower than the Winter wheat in all cases, except in the presence of sunflower extract at 28/14 C and brome grass at 30/15 C. The reason(s) for this is unknown presently but remains a prime topic for future investigation. The role of varying temperatures in the inhibitory activities of Ambrosia artemisiifolia, Ambrosia trifida, Bromus inermis and Helianthus annus were not quite distinct in this study. Perhaps wider temperature ranges would be required before a definite trend is established. Nonetheless, the results revealed that an increased temperature tends to cause increases in inhibitory activity, a finding similar to that of Petinov and Razmaev (1961) who found that higher temperatures slow down the respiration rates in wheat and cotton

plants. It is not possible, at present, to explain the significant temperature-extract interactions found in Winter wheat at both 48 hours and 144 hours. Since temperature did not significantly affect germination of the control seeds, it must somehow either increase the permeability of Winter wheat seeds to the inhibitory agent(s) present in the extracts or promote the interaction of the inhibitory agents with the growth regulatory sites in the wheat seeds. That significant temperature-extract interactions were not found in Spring wheat seed germination too, may be due to genetic differences between the two varieties of wheat, or possibly to the fact that higher extract concentrations may be required to induce these interactions in Spring wheat. Further investigations with varying extract concentrations need to be performed in order to answer the latter proposition.

Effects of Extracts on Wheat Seed Growth and Their Implications

The presence of germination-inhibiting substances in ragweed, brome grass and sunflower has been confirmed by Abdul-Wahab and Rice (1967), Wilson and Rice (1968), and Parenti and Rice (1969). It is believed that phenolic acids present in these plants are partly responsible for their inhibitory activities. Wilson (1970) suggested that phenolic acids affect hormones and growth regulators. The results from the experiment in which seedlings were grown in extracts supported those views, for there was extensive chlorisis in seedlings of both Spring and Winter wheat by the end of the 60 hour period.

The color change was first noticed along the veins, after which it then extended to the entire leaves. It is conceivable, therefore, that seedlings which possess root systems absorb and translocate more of the inhibitory materials than the seeds. Also the seedlings might have absorbed more inhibitory materials since they were grown at a lower temperature (20 C), a finding similar to that of Muruli (1971) who suggested that wheat absorbed more minerals at a lower temperature, 21/15 C, than at a higher temperature of 32/25 C.

The results demonstrate that Winter wheat was more sensitive to aqueous extract inhibition than Spring wheat (Tables I - IV). This may have been due to the genetic differences, or as previously discussed, that higher concentrations of the extracts may be required to produce greater inhibitory responses in that variety of wheat.

From an ecological and economic point of view sunflower, ragweeds and brome grass should not be permitted to grow on the same field that Spring and Winter wheat are growing on because of the fact that they will inhibit and significantly reduce the yield of these valuable crops (Table V). Because the germination period is especially limiting for the successful competition of seedlings, it is necessary that wheat be planted at the proper time so that it can germinate before potential allelopathic plant species become established in the same field. For Spring and Winter wheat to be successfully introduced in the tropics, for example in Nigeria, which is located between the Tropic of Cancer and the equator, and which is also in a belt of warm temperatures, abundant rainfall and year-round solar energy, careful studies must be conducted with regard to temperature and

other plant species which might be inhibitory to the growth and yield of wheat.

SUMMARY

Studies were carried out to determine the effects of varying temperatures between 20 to 30 C day and 10 to 15 C night on the inhibitory activities of aqueous extracts of Ambrosia artemisiifolia (common ragweed), Ambrosia trifida (giant ragweed), Bromus inermis (brome grass) and Helianthus annus (sunflower) on Spring and Winter wheat germination and growth.

The results show that Winter wheat germination in the presence of the extracts was significantly inhibited at temperatures of 24/12, 28/14 and 30/15 C, day/night, than at 20/10 C, day/night, indicating that the wheat absorbed and translocated inhibitory substances more at higher temperatures than at a lower temperature. Although Spring wheat was not affected to the same extent as Winter wheat, a similar pattern was evident, with reduced germination occurring at the higher temperature regimes tested. Inhibitory activities of all the extracts were greatest during the first 48 hours of exposure when the greatest mitotic activity of germination occurred. However, significant reductions in germination in extract-exposed wheat seeds were still evident after 144 hours exposure to the extracts. Dramatic reductions in wheat growth and productivity were also shown in wheat seedlings grown in the presence of the extracts. Only common ragweed-exposed Winter wheat seedlings failed to demonstrate a significant reduction in dry weight after being grown for ten days in the presence of the extract. The data clearly support the contention that these plant species contain phenolic compounds that are inhibitory to wheat seed

germination and growth. Further research will be required in order to isolate the specific compound(s) involved and the mechanisms involved in the production of their inhibitory activities.

LITERATURE CITED

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Abdul-Wahab, A. S., and E. C. Rice. 1967. Plant inhibition by Johnson grass and its possible significance in old field succession. Bul. Torrey Bot. Club 94:486-97.

Borek, E., and H. Waelsch. 1951. The effect of temperature on the nutritional requirement of micro-organisms. J. Biol. Chem. 190:191-196.

Evanori, M. 1949. General inhibitions. Bot. Rev. 15:153-64.

Hoagland, D. R., and D. I. Arnon. 1950. The water culture method for growing plants without soil. Calif. Agr. Exp. Stn. Cir., p. 347.

Jackson, R. J., and W. Willemsen. 1976. Allelopathy in the first stages of secondary succession on the piedmont of New Jersey. Am. J. Bot. 63:1015-23.

Muruli, B. I. 1971. Effect of temperature on uptake and upward translocation of phosphorus in wheat. Master's thesis. ESU, Emporia, Kansas.

O'Donnel, M. S. 1972. A qualitative and quantitative study of the prominent phenolic compounds of Cannabis sativa and its ecological significance. Master's thesis, ESU, Emporia, Kansas.

Parenti, I. R., and E. L. Rice. 1969. Inhibitional effects of Digitaria sanguinalis and possible role in old-field succession. Bull. Torrey Bot. Club, 96(1):70-78.

Paulsen, G. M., and O. A. Rotimi. 1968. Phosphorus-zinc interaction in two-soybean varieties differing in sensitivity to high phosphorus nutrition. Proc. Soil Soc. Amer. 32:73-76.

Pefrnou, N. S., and I. Razmaev. 1961. Effect of high temperatures on respiration rate and carbohydrate metabolism in plants. Fiziologiya Rastenii 8:417-24 (AIBS translation).

Rice, E. L. 1964. Inhibition of nitrogen fixing and nitrifying bacteria by seed plants. Ecology 45:824-37.

Steel, R. G. D., and J. H. Torrie. 1960. Principles and procedures in statistics. McGraw-Hill, New York. 481 pp.

Tendler, M. D., and S. Korman and Nishmote. 1967. Effect of temperature and nutrition on macromolecule production by thermophilic eumycophyta. Bull. Torrey Bot. Club 94:180-89.

Wilson, R. E., and E. L. Rice. 1968. Allelopathy as expressed by *Helianthus annus* in its role in old field succession. Bull. Torrey Bot. Club, 95:432-48.