

# Strategies for Mixed-Grass Prairie Restoration: Herbicide, Tilling, and Nitrogen Manipulation

Scott D. Wilson<sup>1</sup>  
Ann K. Gerry<sup>1,2</sup>

## Abstract

Large areas of North American prairie have been planted with grasses introduced from Eurasia. We examined three strategies (herbicide, tilling, and nitrogen manipulation) for enhancing the establishment of seedlings of native species and suppressing the introduced grasses *Agropyron cristatum* (crested wheat grass) and *Bromus inermis* (smooth brome). Plots (5 × 15 m) were subjected to one of three levels of tilling (none, intermediate, complete) and four levels of nitrogen (none, intermediate, high, and sawdust added to immobilize nitrogen). Treatments were applied in a factorial design with twelve treatments and ten replicates. Seeds of 41 native species were drilled into the plots in May 1992. Following the failure of seeds to establish in 1992, a subplot (5 × 13 m) within each main plot was sprayed with the herbicide glyphosate in April 1993. The nitrogen treatments were repeated in Spring 1993. In August 1993, the density of native seedlings in sprayed subplots was 20 times that in unsprayed subplots. Within sprayed subplots, native seedling density and the cover of bare ground decreased significantly with increasing nitrogen availability. Plots receiving sawdust had significantly higher mean cover of bare ground and significantly lower concentrations of soil available nitrogen. Native seedling density was significantly higher in plots receiving the highest

<sup>1</sup>Department of Biology, University of Regina, Regina, Saskatchewan, S4S 0A2, Canada

<sup>2</sup>Current address: Saskatchewan Conservation Data Centre, 326-3211 Albert St., Regina, Saskatchewan, S4S 5W6, Canada

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intensity of tilling. The responses of native seedlings to all these factors point to the importance of neighbor-free establishment sites as a prerequisite for prairie restoration.

## Introduction

Introduced plant species pose problems for ecological restoration in many systems (Berger 1993). The native vegetation of the North American prairie is frequently replaced with grasses from Eurasia. In western Canada, about 800,000 ha of mixed-grass prairie, naturally dominated by *Bouteloua gracilis* (blue grama) and *Stipa comata* (spear grass), were planted with the introduced grass *Agropyron cristatum* (crested wheat grass) between 1930 and 1970 (Looman & Heinrichs 1973). The resulting vegetation contains few native plants (Hubbard 1949; Looman & Heinrichs 1973; Marlette & Anderson 1986).

There is a growing interest in restoring native species to areas currently dominated by introduced grasses. One of the greatest obstacles to seedling establishment in any habitat is the currently established vegetation (Grubb 1977). The most effective method for removing this obstacle may be treatment with a herbicide such as glyphosate (Grossbard & Atkinson 1985; Malik & Waddington 1990). Alternatively, tilling prior to seeding may provide sufficient time for seedlings to establish before the regrowth of introduced vegetation (Wilson & Tilman 1993). Finally, introduced species are associated with nutrient-rich soils in western Australia (Hobbs & Atkins 1988), California (Huenneke et al. 1990), and Minnesota (Wilson & Tilman 1991), so decreasing soil nutrient availability might favor the establishment of native species (Whitford 1988). Further, decreasing nutrient availability might decrease neighbor mass and increase germination opportunities (Fowler 1988).

Our objective was to examine the influence of herbicide, tilling, and nitrogen manipulation on the establishment of native grasses and the suppression of introduced grasses.

## Methods

The study was conducted in an old field (Fig. 1) at the University of Regina, Regina, Saskatchewan, Canada (50°26'N, 104°40'W). Regina is at the northern edge of the Great Plains and has a continental climate. The average January temperature is -18° C, the average July temperature is 19° C, and the average last and first frosts occur on May 21 and September 12, respectively. The average annual precipitation is 384 mm and falls mostly during thunderstorms in May and June (Environment Canada 1982). The field had not been cultivated and had been dominated by the introduced grasses *Agropyron cristatum* and *Bromus inermis* (smooth brome) for at least 10 years. The soil is dark brown chernozemic (Agriculture Canada 1992). The natural vegetation of the area is mixed-grass prairie.



Figure 1. Part of the study site at Regina, Saskatchewan. At the far right is a 2-meter wide access corridor dominated by the introduced grasses *Agropyron cristatum* and *Bromus inermis*, which dominated the site at the start of the study. At left is a plot that has been sprayed with herbicide and contains bare ground, *A. cristatum*, *B. inermis*, the annual mustard *Thlapsi arvense*, and the native plants *Agropyron trachycaulum* and *Helianthus laetiflorus*.

One of twelve treatments was applied to each of 120 plots (5 × 15 m, separated by 2-meter corridors) in a randomized design. There were 10 replicates of each treatment. The 12 treatments comprised four levels of nitrogen (N1, N2, N3, and N4) and three levels of disturbance (D1, D2, and D3) applied in all combinations (N1D1, N1D2 . . . N4D2, N4D3).

The N1 treatment was designed to reduce the concentration of available nitrogen in the soil. To do this, plots in the N1 treatment were sprinkled with fine sawdust at a rate of 400 g/m<sup>2</sup>/year. This rate was chosen to be in the range of natural primary productivity in the region (Sala et al. 1988). Sawdust was meant to provide a substrate with a high

C:N ratio which would encourage microbes to immobilize available nitrogen (nitrate and ammonium). Plots in the N2 treatment had nothing added, N3 plots received 5 g/m<sup>2</sup>/year of nitrogen, and N4 received 15 g/m<sup>2</sup>/year of nitrogen. The highest nitrogen level (N4) was chosen to approximate agricultural application rates, and the lower level (N3) was chosen to be substantially less. Nitrogen was added in the form of ammonium nitrate in 1992 and urea in 1993. Half the sawdust and nitrogen were applied in early May and the remaining half in mid-June.

Plots receiving the D1 treatment were not disturbed. Plots in the D2 treatment were lightly tilled so that cover of bare ground was about 50% just after tilling. Plots in the D3

treatment were completely tilled so that cover of bare ground was 100%. Disturbance levels were chosen to provide different amounts of bare ground. Plots were tilled to a depth of about 5 cm in late April 1992.

The grass in all plots was mowed to a height of 15 cm in early May 1992 in order to facilitate seeding. After mowing, all plots were seeded with 41 species of native grasses and forbs (Appendix 1) at a rate of 1.936 g/m<sup>2</sup>. Seeds were planted 1–2 cm deep using a native grass drill (Truax, Minneapolis) and packed with a 2-meter-wide roller. Seed masses were chosen on the relative availability, germinability, and natural abundance of each species. Nitrogen treatments in 1992 were applied following seeding.

Few seedlings became established in 1992, so the vegetation in all plots was mowed to 15 cm in height in mid-April 1993 to expose new grass shoots, and the northern 5-by-13-meter portion of each plot was sprayed with 10 liters/ha glyphosate, a rapidly decomposing, systemic herbicide. Glyphosate was applied before native seed germination. The 5-by-2-meter area at the south end of each plot was not sprayed. In summary, two herbicide treatments (sprayed or not) were applied to the 12 combinations of nitrogen and disturbance.

Vegetation was sampled during August 16–20, 1993, from a quadrat (50 × 100 cm) centered in each unsprayed subplot and from a second quadrat in each sprayed subplot, 2 meters north of the first quadrat. The seedlings of each native species were counted, and the covers of all species and bare ground were recorded using Daubenmire's scale. Split-plot analysis of variance (ANOVA) was used to examine the effects of herbicide, with disturbance and nitrogen as the main plot effects and herbicide as a subplot effect. Almost no native seedlings were found in quadrats not sprayed with herbicide, so these quadrats were excluded from further analysis. The influence of nitrogen and disturbance in quadrats receiving herbicide were examined using two-factor ANOVA. Proportional data (such as species covers) were arcsine-square-root-transformed, and other data were log<sub>10</sub>-transformed to reduce heteroscedasticity and improve normality. Untransformed data are presented.

Soil available nitrogen (sum of ammonium and nitrate) was measured in unsprayed subplots of undisturbed plots on June 1, 1993, when the early summer rainy season causes a peak in soil nitrogen dynamics (Wilson 1993). We measured only this subset of treatments because we particularly wished to examine the effect of sawdust addition on nitrogen availability under undisturbed introduced grasses. Four soil cores (2 cm diameter, 10 cm deep) were taken from each plot and combined, and a 10-gram subsample was extracted for 2 hours in 0.02 M KCl. The solution was then decanted and frozen until analysis with an ion-selective electrode. ANOVA tested whether available soil nitrogen varied significantly among nitrogen levels.

## Results

**Herbicide Effects.** Seven seedlings of native species were found in August 1993 in the 120 quadrats not sprayed with herbicide (Table 1). Many more seedlings were found in quadrats sprayed with herbicide, mostly the grasses *Andropogon gerardii* (big bluestem) and *Agropyron trachycaulum* (slender wheat grass; Table 1). The total density of native seedlings was analyzed because of the low abundance of individual species. Native seedling density was significantly higher ( $p < 0.05$ ) in sprayed quadrats than in unsprayed, as was the cover of bare ground (Table 2).

Unsprayed quadrats were dominated by the introduced perennial grasses *Agropyron cristatum* and *Bromus inermis* (Table 3). The total cover of introduced perennial grasses was significantly lower in quadrats sprayed with herbicide ( $F = 773.4$ ,  $p < 0.001$ ). The native shrub *Rosa arkansana* (prairie rose) was sparse (0.1% cover) but was the third most common species in unsprayed quadrats. *Rosa arkansana* occurred in the field prior to treatment application because it persists in agricultural fields. Sprayed quadrats were dominated by annual weeds (Table 3). The total cover of annual weeds was significantly higher in quadrats sprayed with herbicide (19%) than in unsprayed quadrats (0.5%;  $F = 265.9$ ,  $p < 0.001$ ).

**Nitrogen and Disturbance Effects in Sprayed Quadrats.** In quadrats sprayed with herbicide, the density of native species varied significantly with both nitrogen and disturbance but not with the interaction between them. Density of native seedlings decreased significantly with increasing nitrogen ( $F = 7.44$ ;  $p < 0.001$ ; Fig. 2). Plots receiving sawdust or no nitrogen (N1 and N2 treatments) had significantly higher densities of native species than plots receiving the highest rate of nitrogen application (N4). The only treatment that produced no native seedlings was N4 with no disturbance (N4D1; Fig. 2). Density of native seedlings also varied among disturbance treatments ( $F = 6.03$ ;  $p < 0.01$ ; Fig. 2). Comparisons among disturbance treatments across all nitrogen levels showed that plots with intermediate levels of dis-

**Table 1.** Total numbers of seedlings of native species found in quadrats (total area 60 m<sup>2</sup>) dominated by introduced grasses that were either not sprayed or sprayed with herbicide, at Regina, Canada.

Species	Not Sprayed	Sprayed
<i>Andropogon gerardii</i>	4	93
<i>Agropyron trachycaulum</i>	0	27
<i>Ratibida pinnata</i>	1	11
<i>Petalostemon purpureum</i>	1	6
<i>Potentilla pensylvanica</i>	0	4
<i>Helianthus laetiflorus</i>	1	2
<i>Bouteloua gracilis</i>	0	1

**Table 2.** Mean density of native seedlings and mean cover of bare ground in quadrats either sprayed or not sprayed with herbicide ( $n = 120$ ).

	Not Sprayed	Sprayed	F*
Native species (plants/m <sup>2</sup> )	0.12	2.4	82.8**
Bare ground (% cover)	34	59	185.0**

\*F value for spray treatment from analysis of variance.

\*\*Means are significantly different ( $p < 0.001$ ).

turbance (D2) had lower densities of native species than did undisturbed (D1) or highly disturbed (D3) plots.

The cover of bare ground decreased significantly with increasing nitrogen ( $F = 15.43$ ;  $p < 0.001$ ; Fig. 3). Plots receiving no nitrogen (N2) and those receiving the smallest amount of nitrogen (N3) did not differ in bare ground, but plots receiving sawdust (N1) had significantly more bare ground, and those with the highest rate of nitrogen addition (N4) had significantly less bare ground (Fig. 3). Bare ground did not vary significantly with disturbance.

The total cover of introduced perennial grasses did not vary significantly with either nitrogen or disturbance (Fig. 4). Similarly, the total cover of nonplanted annuals did not vary with either nitrogen or disturbance (Fig. 5).

There was no significant interaction between nitrogen and disturbance for any variable.

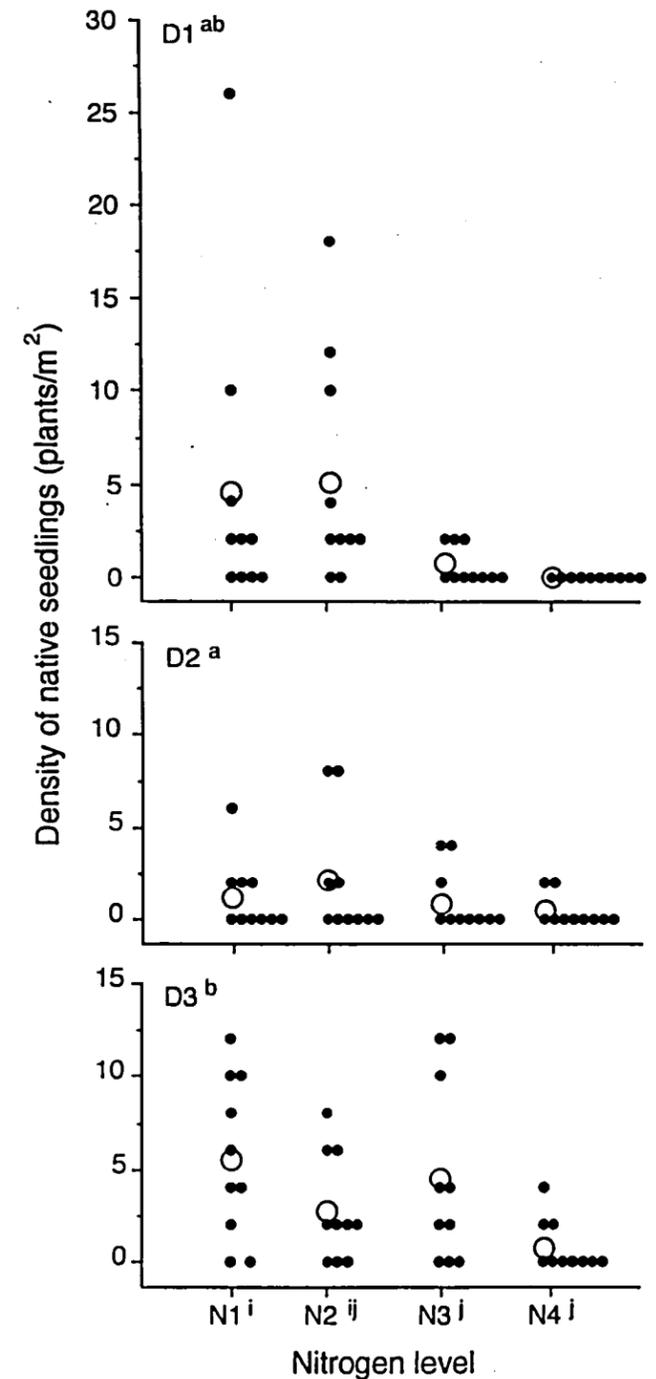
**Soil Nitrogen.** Available soil nitrogen varied significantly among nitrogen levels in unsprayed, undisturbed vegetation (N1: 0.07 mg N/kg soil; N2: 0.68; N3: 4.15; N4: 16.65;  $F = 208.9$ ,  $p < 0.001$ ). Pairwise comparisons showed that added nitrogen significantly increased soil nitrogen availability over control plots and that sawdust addition significantly decreased soil nitrogen availability.

## Discussion

Grassland retired from cultivation or recovering from other disturbance appears to revert to native species (Dormaar

**Table 3.** Mean covers (%) of common nonplanted species (> 1%) and totals for perennial grasses and annuals in quadrats either sprayed or not sprayed with herbicide.

Species	Not Sprayed	Sprayed
<b>Perennials</b>		
<i>Agropyron cristatum</i>	35.7	4.5
<i>Bromus inermis</i>	31.6	9.1
<i>Rosa arkansana</i>	0.1	2.0
Total Perennial Grasses	67.3	13.6
<b>Annuals</b>		
<i>Euphorbia serpyllifolia</i>	0.2	10.7
<i>Cirsium arvense</i>	0.2	3.8
<i>Thlaspi arvense</i>	0.0	4.2
Total Annuals	0.5	19.1



**Figure 2.** Density of native seedlings in quadrats sprayed with herbicide and subjected to three levels of disturbance (D1: not tilled; D2: lightly tilled; D3: completely tilled) and four levels of nitrogen (N1: sawdust added; N2: nothing added; N3: 5 grams N/m<sup>2</sup>/yr; N4: 15 grams N/m<sup>2</sup>/yr). Points represent data from individual plots. Circles represent treatment means.<sup>a,b</sup> Disturbance treatments with common superscripts are not significantly different ( $P > 0.05$ ).<sup>i,j</sup> Nitrogen treatments with common superscripts are not significantly different.

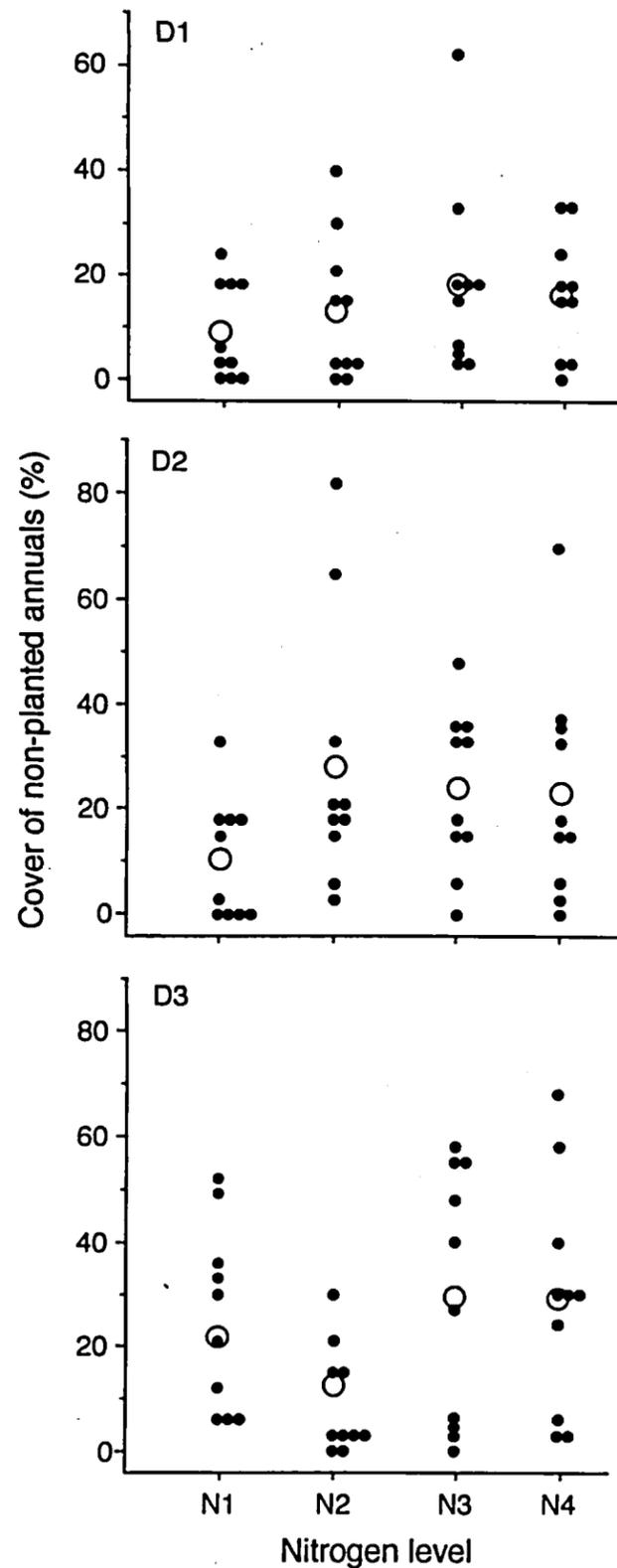


Figure 5. Cover of nonplanted annuals in quadrats sprayed with herbicide and subjected to three levels of disturbance (D1: not tilled; D2: lightly tilled; D3: completely tilled) and

grasses in sprayed subplots did not vary with nitrogen addition (Fig. 4, 5), but this may be because the experiment was sampled at the end of the summer when plant growth had obscured any seasonal differences among treatments (Wilson & Tilman 1993).

This experiment continues and will allow us to monitor the long-term fate of native seedlings and the control of introduced grasses. Some introduced grass persisted in the sprayed subplots (Fig. 1, Table 2) and may require further herbicide treatment. Herbicide would need to be applied selectively to the introduced grasses, possibly using a wick applicator. A possible modification to the treatments is an increase in the rate of sawdust addition. The current rate increased bare ground (Fig. 3) and decreased nitrogen availability but did not increase native seedling density (Fig. 2).

In summary, treating introduced grasses with herbicide resulted in a 20-fold increase in the density of native seedlings. Tilling without herbicide treatment resulted in very low establishment of native seedlings. Within sprayed plots, adding nitrogen decreased bare ground and native seedling density. Sawdust increased the cover of bare ground. Tilling increased seedling density. The responses of native seedlings to all these factors points to the importance of neighbor-free establishment sites as a prerequisite for prairie restoration.

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four levels of nitrogen (N1: sawdust added; N2: nothing added; N3: 5 grams N/m<sup>2</sup>/yr.; N4: 15 grams N/m<sup>2</sup>/yr.) Points represent data from individual plots. Circles represent treatment means.

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Appendix 1. Native species sown in May 1992 and the total mass of seeds sown in the 1.003-ha study site (area includes plots and some access corridors).

Scientific Name	Common Name	Mass (g)
<b>Grasses</b>		
<i>Agropyron trachycaulum</i>	slender wheatgrass	4500
<i>Agrostis scabra</i>	hair grass	2500
<i>Andropogon gerardi</i>	big bluestem	2500
<i>Avena fatua</i>	wild oats	trace
<i>Bromus kalmii</i>	fringed brome	500
<i>Bouteloua gracilis</i>	blue grama	2500
<i>Elymus canadensis</i>	Canada wild rye	200
<i>Panicum capillare</i>	witch grass	50
<i>Panicum virgatum</i>	switch grass	100
<i>Schizachyrium scoparium</i>	little bluestem	2000
<i>Setaria viridis</i>	green foxtail	trace
<i>Sporobolus heterolepis</i>	prairie dropseed	200
<b>Forbs</b>		
<i>Achillea millefolium</i>	yarrow	2
<i>Anemone cylindrica</i>	long-fruited anemone	20
<i>Anemone multifida</i>	cut-leaved anemone	20
<i>Anemone patens</i>	prairie crocus	trace
<i>Artemisia frigida</i>	pasture sage	10
<i>Aster ericoides</i>	many-flowered aster	220
<i>Aster laevis</i>	smooth aster	440
<i>Astragalus canadensis</i>	Canada milkvetch	25
<i>Echinacea angustifolia</i>	purple coneflower	10
<i>Gaillardia aristata</i>	gaillardia	50
<i>Geum triflorum</i>	three-flowered avens	44
<i>Helianthus laetiflorus</i>	rhombic-leaved sunflower	60
<i>Helianthus maximiliani</i>	narrow-leaved sunflower	100

Continued

Appendix 1. Continued

Scientific Name	Common Name	Mass (g)
<i>Heliopsis helianthoides</i>	rough false sunflower	40
<i>Liatris ligulistylis</i>	meadow blazingstar	80
<i>Liatris punctata</i>	dotted blazingstar	44
<i>Linum lewisii</i>	wild flax	10
<i>Monarda fistulosa</i>	bergamot	2
<i>Oenothera biennis</i>	yellow evening primrose	20
<i>Petalostemon purpureum</i>	purple prairie clover	660
<i>Potentilla pensylvanica</i>	prairie cinquefoil	10
<i>Ratibida pinnata</i>	yellow coneflower	100
<i>Rudbeckia hirta</i>	black-eyed susan	5
<i>Solidago canadensis</i>	Canada goldenrod	120
<i>Solidago missouriensis</i>	low goldenrod	25
<i>Solidago nemoralis</i>	showy goldenrod	66
<i>Solidago ptarmicoides</i>	white goldenrod	5
<i>Solidago rigida</i>	stiff goldenrod	110
<i>Zizia aptera</i>	heart-leaved alexander	30