DISSERTATION

WILD PROSO MILLET (*Panicum miliaceum* L.) BIOTYPES: GROWTH ANALYSIS, COMPETITIVE ABILITY, AND GENETIC VARIATION

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY DÉCIO KARAM ENTITLED **WILD PROSO MILLET (*Panicum miliaceum* L.) BIOTYPES: GROWTH ANALYSIS, COMPETITIVE ABILITYs AND GENETIC VARIATION** BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

WILD PROSO MILLET (PANICUM MILIACEUM L.): GROWTH ANALYSIS, COMPETITIVE ABIILITY, AND GENETIC VARIATION

Greenhouse and laboratory experiments were conducted with three domestic and nine wild proso millet (*Panicum miliaceum* L.) biotypes. Growth during 70 days after planting was evaluated using the Richards function, which characterized relative differences in growth. Significant differences in shoot dry weight and leaf area expansion were observed for proso millet biotypes. The average shoot dry weight RGR was 0.095 g g^{-1} day^{-1}. AGR of shoot dry weight ranged from 0.24 to 0.45 g day^{-1}. Proso millet biotypes significantly differed in leaf area 26 DAP. Shoot dry weight and leaf area RGR did not differ among biotypes while differences were observed for AGR. Proso millet biotypes did not differ for specific leaf area although differences were detected for leaf area ratios. Domestic biotypes were shorter than wild biotypes. Tiller number varied from 2 to 4 per plant. Plant biomass partitioning results indicate different pattern of dry weight accumulation. Reproductive development started between the third and fourth week after planting, depending on biotype. Seed germination ranged from 28 to 100% 14 days after incubation. Seed coat as a percent of total seed biomass varied between 12.2 and 29.8%. Black seeded biotypes had different germination rates that ranged from 17 to 91% but no differences were observed for seed coat percent and seed weight. Shoot dry weight, leaf area, and biomass partitioning early growth differed among biotypes. The competitive ability detected between Canada-Rosemount black seeded and Colorado-Weld County tan seeded biotypes suggests a greater competitive ability for the Canada biotype. The genetic variation based on AFLP technique detected two distinct groups: (i) all domestic biotypes with black and olive seeded wild biotypes and (ii) tan seeded biotypes with a Wyoming brown seeded wild biotype. The genetic diversity observed among biotypes may correlate with differences in growth and competitive ability observed in these experiments. Although the molecular biology DNA research was conducted last in this thesis, future research of this type should begin with DNA assessment to detect the

most diverse domestic and wild biotypes. This would allow for more focused research on growth analysis and competition studies.

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CHAPTER 1

LITERATURE REVIEW

Proso millet (*Panicum miliaceum* L.)

BOTANICAL DESCRIPTION

Panicum miliaceum L., proso millet, is an annual C_4 grass family Poaceae, subfamily Panicoideae and tribe Paniceae. According to Häfliger and Scholz (1980) proso millet as a weed is commonly called wild proso millet (U.S.A.), broomcorn millet (New Zealand), millet panic (Australia), millo (Cuba), and panic faux millet (France). It is a warm-season grass (Anderson and Nielsen 1996), usually 30 to 100 cm tall (Purseglove 1972, Bought et al. 1986) but frequently taller (200 cm) as a weed (Stubblendieck et al. 1995) cm tall. The root system is shallow (Bough et al. 1986). It has erect glabrous or slightly pubescent culms (stems) strongly branching at the base (Bough et al. 1986, Stubblendieck et al. 1995). Culm diameter ranged from 4.3 to 8.7mm in fifteen wild proso millets from the United States and Canada (Westra and Callan 1990). Flat linear lanceolate leaves (5-40cm long, 6-20mm wide) are pubescent or without hair on both sides (Häfliger and Scholz 1980, Stubbendieck et al. 1995). The ligule is a short-fringed ciliate membrane measuring from 1 to 3mm long (Stubbendieck et al. 1995). The leaf sheaths are rounded pubescent on lower leaves or pubescent on the sheath-margin or sometimes pubescent only at the summit (Häfliger and Scholz 1980, Stubblendieck et al. 1995).

Panicles (10- 40cm) are erect or nodding at maturity, usually open but sometimes contracted depending on population type (Häfliger and Scholz 1980, Stubblendieck et al. 1995). Wild and cultivated proso millet produces panicles at 46 and 32 days (Carpenter and Hopen 1985). The spikelets that range from 4.5 to 5.3mm long and 1.7 to 2.3mm wide (Purseglove 1972, Häfliger and Scholz 1980), are solitary pedicelled with 9 to 15 nerves (Stubbendieck et al. 1995). The sharply pointed glumes are unequal. The first with 7 to 11 nerves is half as long as the spikelets while the second with 11 to 13 nerves equaling the spikelets (Häfliger and Scholz 1980, Stubbendieck et al. 1995). The lemma and glumes remain around the caryopsis after it is shed (Bough et al. 1986).

P. miliaceum is classified as self-pollinated but at least 10% cross-pollination may occur (Popov 1947). Warwick (1990) reported the number of chromosomes for five selffertilizing weeds of maize and soybean monocultures where *P. miliaceum* has 36 chromosomes (Bajaj 1981, M'Ribu and Hilu 1994). Reproduction is by seeds where a range of seed color can be observed. Seed maturation can vary from 60 to 70 days for some crop varieties but 10 to 14 days latter in maturity can be observed (Croissant and Shanahan 1992). Seed color varies from light cream to almost black and they may shatter easily dispersing over time or may be retained on the panicle until the end of the growing season (Bough et al. 1986, Bough and Cavers 1987). Seed size ranges from 2.5 to 3.0mm long and 1.5 to 2.0mm wide (Doersch et al. undated, Purseglove 1972, Miller and Whitson 1986). Seed mean weight for weed biotypes varies from 3.69 to 3.75mg/seed while for croplike types it varies from 4.90 to 6.23mg/seed (Moore and Cavers 1985). Cavers and Bough (1985) observed variation in seed weight from 4.7 to 6.2mg/seed in a study where cultivated proso millet was compared with weedy proso millet. Miller and Whitson (1986) state that in infested areas, wild proso millet can produces 300 or more seeds per square foot while Doersch and coworkers (undated) state that a single plant frequently can produce 2,000 or more seeds.

According to Bough et al (1986) and Bough and Cavers (1987) the best way to distinguish proso millet from similar grasses is the seed hull or husk that remains affixed to the root until maturity. Two other *Panicum* can be confused with proso millet: *Panicum dichotomiflorum* Michx. and *Panicum capillare* L.. *P. dichotomiflorum* (fall panicum) is a shorter plant with a similar panicle and smaller seeds. *P capillare* (grass witch) also is a shorter plant with an open spreading panicle where seeds are smaller and lighter (Bough et al. 1986, Subbendieck et al 1995).

ORIGIN AND LOCATION

According to Youtai (1987) the "Five Grains", a Chinese expression to explain the first priority in Chinese agriculture first appeared in the 85 BC. The five grains were millet, glutinous millet, soybean, wheat, and rice. "Shu" and "Ji" leaders of the "Five Grains" are *P. miliaceum* and a variety of "Shu" respectively. Seeds of the two species were found in excavated relics of the New Stone Age evidence of a six to seven thousand year history.

In 1949, Anderson and Martin reported that millet was used as food in prehistoric periods but little was known about its origin. Egypt-Arabia is probably the origin of millet Candolle 1964)

Anderson and Martin (1949) Grabouski (1971), and Baltensperger (1996), state that millets have been grown since ancient times in China, Egypt, India, Russia, Southern Europe, and Japan. It is currently produced in Eastern Europe, Russia, China, India, and North America. Marinval (1992) reported a study of the archaeobotanical data on millets including proso millet in France. The oldest evidence in France dates from about 3500 BC.

Proso millet provides food for mourning doves, pheasant, quail, and songbirds (Stubbendieck et al. 1995). Although the primary use of proso millet is bird and livestock feed (Nelson 1984), the grain is still used in many parts of the world as a human food source. In the Great Plain areas of western Nebraska, northern Colorado, South Dakota, and North Dakota proso millet has been grown for livestock feed and birdseed (Grabouski 1971).

In 1980, Rachie and Majmudar stated that proso millet was grown mainly in the USSR and eastern Asia which produced about 88% of the total world production. The Consultative Group on International Agricultural Research[1] (1999) reports that Asia and Africa account for about 94% of the global production of millets estimated to be

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[1] Consultative Group on International Agricultural Research. 1999. http://www.cgiar.org

approximately 28 million tons/year for 1992-1994 periods. Of the global millet production, proso millet participates with 15%.

In the last 25 years, wild-proso millet, the weedy form of proso millet, has become one of the most aggressive grass weeds in North America. Infestations of this weed were first reported in Minnesota and Wisconsin in the early 1970s (Strand et al. 1973, Cavers and Bough 1985; Doersch and Harvey 1979, Harvey 1979, Westra et al. 1990). Since then, wild-proso millet has become a problem in different regions of the United States (Westra et al. 1990) and Canada (Cavers et al. 1985). It causes yield losses in corn in Colorado, Idaho, Minnesota, Montana, North Dakota, Oregon, South Dakota, Utah, Wisconsin and Wyoming (WSSA 1992). Wild proso millet is also weedy in potato, sugarbeets, wheat, and soybean.

According to Westra[2] (1997) corn yield losses due to wild proso millet have been estimated to be more than 2 million dollars annually in Colorado and more than 50 million dollars annually in the United States and Canada.

[2] Westra, P. 1997. Personal communication. Colorado State University, Ft. Collins, CO 80523

BIOTYPES

Although many experiments have been done with wild-proso millet, few experiments have demonstrated biotypic responses. Biological characteristics of many different proso millets were described by Bough et al. (1986). Khan et al. (1996) reported the role of the pigmented seed coat of proso millet biotypes in inhibition, germination and seed persistence. Because darker seeds had heavier seed coats, inhibited and germinated more slowly, and suffered less inhibition damage, the authors stated the importance of those factors increase persistence in the soil of the darker-seeded weedy biotype.

Eberlein et al. (1990), studying growth and development of three wild-proso millet biotypes and one cultivated proso millet, verified that seed production was 1.4 to 2.0 times greater for wild than cultivated proso millet. They also observed differences in growth characteristics including number of leaves in the main culm, and length and width of flag leaf. When reproductive characteristics were analyzed, the authors verified differences in panicle size, seed color, seed germination, and seed shattering.

Comparing the biology of wild and cultivated proso millet, Carpenter and Hopen (1985) found that plant height of both wild and cultivated proso millet increased linearly as the length of photoperiod increased. Cultivated proso millet was taller and accumulated more dry matter than wild-proso millet. Warwick and Thompson (1987), in a similar study, observed that variation in total biomass was reduced at increasing density of wild proso millet. In the same study they verified that white (large white crop type) and golden (golden type) seeded biotypes, in inter-biotype interaction, had greater competitive ability than orange, crown, and black biotypes.

Seven biotype characteristics of *P. miliaceum* were evaluated by Bough et al. (1986) for origin, seed color, seed size, seed weight, seed dormancy, time to maturity, tillering, herbicide tolerance, panicle shape, and shattering. Seed size varied from 2.5 to 3.0mm in length and from 1.5 to 2.25mm in width. Mean weight per seed ranged between 3.0-4.2mg in the middle season for black seeded and 6.5mg for Elgin (light golden seeded biotype) biotype. Differences in seed dormancy were observed varying from little for golden, crown, orange-red to strong for black, and dark-red seeded biotypes.

Moore and Cavers (1985) utilized three groups of proso millet varying in seed size and color to compare seedling vigor and germination rates. The results showed that the crops and croplike seeds have greater seedling vigor than wild types. Seed dormancy was a more important aspect of wild-proso millet than seed size in determining germination rate. Cultivated proso millet had faster and higher germination rate.

Studying the role of the seed coat pigmentation of eleven *P. miliaceum* in water uptake, inhibition damage, seedling growth, and seed persistence in soil, Khan et al (1996) showed that dark-pigmented seeds had slower germination rates than light colored seeds. White pigmented seeds took 36 hours to achieve maximum germination while black pigmented seeds took 108 hours. Light seeded biotypes imbibed faster than dark seeded biotypes. The greater inhibition damage for dark seeds was due to the heavier seed coat observed.

Black/olive brown seeds, described by McCanny et al. (1988) as the most aggressive *P. miliaceum* biotype in Ontario, shattered when ripe and was dormant when shed. Other biotypes retain their seeds on the inflorescence, but other biotypes have little or no dormancy when shed. In Canada, golden seed biotypes appear in herbarium records dated prior to black seed biotypes. Black and golden seeded biotypes, according to their studies, were similar in their ability to persist as weeds. The black seeded biotype showed greater potential to colonize uninfested fields due to the potential to spread seeds rapidly.

McCanny and Cavers (1988) demonstrated that black seeded biotypes are more likely to be carried into an uninfested field by a combine than the golden biotype. On average, 3.3% of black seeds were carried more than 50m compared to 0.9% of golden seeds.

Black seeded biotypes are expected to survive better than golden seeded biotypes, because the former biotype has an enhanced ability to survive the winter in the soil (Colosi et al. 1988).

Swanton and Chandler (1990) evaluated imazethapyr, an imidazolinone herbicide, against seven biotypes of wild-proso millet found in Ontario and Quebec. Although there was no significant difference in shoot dry weight with increasing doses of imazethapyr at 3 to 5 leaf growth stage, black-seeded wild-proso millet showed more tolerance to the herbicide at 5 to 7 leaf growth stage than other biotypes.

INTERFERENCE

Several researchers have observed responses to competition between wild-proso millet and crops. Wilson and Westra (1991), conducted field experiments and observed corn yield reduction from 13% to 22% when corn was in competition with 10 wild-proso millet per square meter. Ten plants wild proso millet growing with corn produced between 4,200 and 6,200 seeds per square meter depending on the location where the experiment was conducted.

Wilson (1993) evaluated the effect of wild-proso millet interference in dry beans over a 2-year period in Nebraska. Dry bean yield was reduced from 12 to 31% in a plot with 10 plants of wild-proso millet per square meter.

P. miliaceum density significantly reduced height, fruits per plant, fruit weight, shoot weight and total weight of triazine-resistant *Brassica napus*. *B. napus* fruit weigh was reduced at 200 *P. miliaceum* per square meter, fruit number and shoot weight were inhibited at 400 proso millet m^{-2}, and height was reduced at 600 weeds m^{-2}. Flowering delay was observed at 600 weeds per square meter (Miller and Callihan 1995).

MANAGEMENT

According to Rodriguez et al. (1990) there was no available information on primary, secondary and micronutrient uptake for proso millet prior to 1990. Evaluating dry matter and nutrient accumulation they observed that total dry matter accumulation followed a sigmoidal pattern. Emergence increased linearly to maturity and then progressed at a diminishing rate to leaf senescence during ripening. At physiological maturity the stalks, leaves, and inflorescence accounted for 34, 11, and 55% of the total dry matter production respectively. N, P, and S concentrations decreased in leaves as plants approached maturity while Ca and Mg concentrations increased. Fe concentration remained constant over time while Cu decreased as the plant approached maturity. Sixty days after planting, proso millet accumulated 23.33g kg^{-1}, 2.7 kg^{-1}, 31.0g kg^{-1}, 3.3g kg^{-1}, 4.3g kg^{-1}, 2.6g kg^{-1}, 212.2mg g^{-1}, 47.8mg g^{-1}, 21.5mg g^{-1}, and 14.8mg g^{-1} of N, P, K, Ca, Mg, S, Fe, Mn, Zn, and Cu respectively.

Wiese and Binning (1987) calculated the low threshold temperature of development for *P. miliaceum*, *Echinochloa crus-galli* (barnyardgrass), *Chenopodium album* (lambsquarters), and *Amaranthus retroflexus* (redroot pigweed). The regression line adjusted indicated 6.9°C as the low threshold temperature for germination of wild proso millet.

The allelopathic potential of residues and aqueous extracts of rye (*Secale cerealle*) was evaluated on the germination and seedling growth of four plant species (Barnes and Putnam 1986). *P. miliaceum* emergence was inhibited by different tissue residues. Root length was reduced 17% in the presence of 1.0g of root tissue and 41% by 1.0g of shoot tissue, respectively. When residues were combined growth was reduced by 73%.

Westra et al. (1990) evaluated seven weed management treatments that successfully controlled more than 88% of the wild-proso millet until harvest. These treatments resulted in a 68% increase in average corn yield compared to untreated control.

Eight herbicide treatments controlled proso millet greater than 87% in processing peas (*Pisum sativum* L.) while in early season soybean (*Glycine max* (L) Merr.), ethalfuralin (0.8kg ha^{-1}), fluchloralin (1.7kg ha^{-1}), pendimethalin (0.8kg ha^{-1}), profluralin (1.7kg ha^{-1}), and trifluralin (0.8kg ha^{-1}) controlled more than 85%. The late season wild proso millet control ranged from 4 to 88% with trifluralin at 1.7kg ha^{-1}; the only herbicide to control above 85% (McNevin and Harvey (1982).

Uptake, metabolism and activity of haloxyfop in liquid cultures of proso millet were reported by Irzyr et al. (1990). Haloxyfop was toxic to proso millet cells at all growth stages studied. Fresh weight inhibition (IC_{50}) was 0.23, 74, 158, and 74μM for 1, 4, 7, and 10 days culture, respectively. Although after 4-days cells absorbed greater amounts of haloxyfop than 1-day cells. In the first hour, 1-day and 4-days cell absorbed more than 60% of applied haloxyfop.

Harvey and McNevin (1990) combined cultural practices and herbicides to control wild proso millet. Benefin and EPTC reduced weed biomass in alfalfa (*Medicago sativa* L.). The combination of herbicide and crop forage harvest prevented weed seed production. Wild proso millet seedling populations were reduced and corn yield increased when corn followed 1 to 4 years of alfalfa. Corn planted in alfalfa mulch with application of pendimenthalin (2.2kg ha^{-1}) plus simazine (2.2kg ha^{-1}) or pendimenthalin (2.2kg ha^{-1}) plus cyanazine (2.2kg ha-1) provided the best wild proso millet control. Several postemergence herbicides were also evaluated in new seedling alfalfa (Miller 1986 a, 1988a).

After evaluating different herbicides for 6 years in field experiments, Harvey and Porter (1990) observed that only CGA-82725 at 0.56kg ha^{-1} when applied between 20 and 28 days after planting provided 90% wild proso millet control in the early July. Those herbicides applied from 33 to 36 days after planting, haloxyfop at 0.07 and 0.14 kg ha^{-1}, sethoxydim at 0.11 and 0.17 kg ha^{-1}, fluazifop at 0.28 kg ha^{-1}, and CGA-82725 at 0.28 and 0.43 kg ha^{-1} controlled from mid August to early September between 88 and 96% of wild proso millet

Shenk et al. (1990) identified chemical control programs to control wild proso millet. Their results indicated that only EPTC (4.5kg ha^{-1}) or vernolate ((6.89kg ha^{-1}) with a protectant applied preplant incorporated, or alachlor (4.5kg ha^{-1}) preemergence followed by atrazine (1.7kg ha^{-1}) plus tridiphane (0.84kg ha^{-1}) postemergence controlled wild proso millet more than 85%.

Several other experiments have evaluated the effect of herbicides on wild-proso millet. These studies have been conducted in different regions under different conditions. Harvey and Porter (1990) evaluated several postemergence herbicides applied in soybeans. Strand et al. (1973), Curtis et al. (1989), Miller et al. (1989), Miller and Shoemak (1986), Miller (1986 b, 1987, 1988b), Kleppe and

Harvey (1991), Fawcett and Harvey (1988), Doersch and Harvey (1979), Ezra et al. (1985), Downard and Morishita (1992), and Westra and Stump (1992) evaluated wild proso millet control with herbicides in corn. McGrath et al. (1988) evaluated sethoxydim in snapbeans for wild proso millet control.

GENETIC DIVERSITY

Yashovskii (1975) investigated the nature of the inheritance of millet seed color. The grain color is controlled by the interaction of a system of structural genes and a system of regulatory genes. It was assumed that the dark seed coat appeared as a consequence of the simultaneous genes controlling the synthesis of yellow, red and dark pigment.

The first analysis using techniques in molecular biology to evaluate genetic diversity of proso millet was done by M'Ribu and Hilu in 1994. Interspecific and intraspecific variation in different accessions of *P. miliaceum* was detected through RAPD. A high degree of variability in RAPD markers was observed with approximately 73% of 199 markers being polymorphic implying high genetic polymorphism.

Colosi and Shaal (1997) found 97 RAPD genotypes (69 genotypes of wild proso millet, 26 genotype of crop cultivars and crop-like weeds, and 2 hybrids between wild proso millet and crop cultivars) among 398 individuals. The result, in about 10% of the genotypes, suggests hybridization between wild and crop biotypes.

Warwick (1987) evaluated isozyme variation in 110 seed accessions of proso millet. Although the results have revealed very low levels of allozyme polymorphism, individuals have displayed considerable fixed heterozygosity for a range of isozymes.

Combination of genetic and isozyme studies have been conducted. Taniguchi et al. (1992) isolated cDNA for isozymes for aspartate aminotransferase (AspAT) in proso millet and the gene structure that encodes those isozymes. Was investigated by Taniguchi et al. (1994)

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CHAPTER 2

WILD AND DOMESTIC PROSO MILLET (*Panicum miliaceum* L.) BIOTYPES: GROWTH ANALYSIS AND SEED GERMINATION

ABSTRACT

Three domestic and nine wild proso millet biotypes were studied at Colorado State University. Growth analysis was conducted for 70 days after planting (DAP) at 14 days intervals starting 14 DAP. Plant height, tiller number, dry weight (leaf, stem, root, and panicle), and leaf area were recorded. Relative growth rate (RGR) and Absolute growth rate (AGR) were derived from the Richards function fitted to shoot dry weight and leaf area. Leaf area ratio (LAR) and specific leaf area (SLA) were fitted, over time, using an exponential regression. Percent of total biomass accumulation was evaluated by a second-degree polynomial equation, and plant height by a logarithmic curve. Differences among biotypes were observed in all parameters. Shoot dry weight was the only parameter to show different pattern of accumulation between two biotypes over all

experimental periods. In general, at the last harvest, Colorado-Weld County tan seeded showed the highest values measured. Shoot dry weight ranged from 13.11g to 26.31g. Shoot and leaf area RGR averaged 0.095 g $g^{-1}day^{-1}$ and 0.062 cm^2 $cm^{-2}day^{-1}$, respectively. Mean shoot AGR ranged from 0.24 to 0.45 g day^{-1}. Mean leaf area AGR varied from 10.90 to 21.91 cm^2 day^{-1}. LAR and SLA were not different among biotypes. Colorado-Weld County tan seeded biotype allocated only 6% of total dry matter to reproductive tissue, the lowest value found. Domestic and wild proso millet heights were 130 and 145 cm, respectively. Biotypes also had different numbers of tillers ranging from 2.1 to 3.5 tillers/plant. Seed color, germination, radicle length, seed coat, and seed weight were determined. Germination varied from 17 to 100% depending on the biotype. Seed coat as a percentage of the total seed weight ranged from 12.2% to 28.4%. Seed weight varied from 5.3 and 7.5mg per seed. Domestic proso millet had greater radicle length than wild biotypes. Early growth was studied for 18 days after planting (DAP). Plant height, leaf area, leaf and shoot dry weight were recorded for 14 days every day after emergence. Leaf, root and stem weight ratio were determined 11 and 18 DAP. Biotypes of wild and cultivated proso millet showed different early growth with different weight ratios.

Canada-Rosemount a black seeded biotype had the lowest values.

INTRODUCTION

Proso millet (*Panicum miliaceum* L.), one of the oldest cereals in the world (Anderson and Martin 1949, Grabouski 1971, Baltensperger 1996), is a crop and a weed (Yashovskii 1974,Cavers and Bough 1985, Moore and Cavers 1985, Bough et al. 1986, Westra and Callan 1990, Colosi and Shaal 1997). Although little is known about its origin, Egypt-Arabia has been indicated as the possible region where it was first observed (Candolle 1964, Matz 1986). Introduced to North America in the 17th century (Grabouski 1971, Bough and Cavers 1987), it has been used there mainly as food source for bird and livestock (Grabouski 1971, Nelson 1984). Proso millet also is used as human food by many Asian and African people (Cardenas et al. 1984). In the United States, infestation of wild proso millet was first described in the early 1970's (Strand et al. 1973, Doersch and Harvey 1979, Harvey 1979), and was spread to over 400,000 ha of Colorado, Idaho, Illinois, Minnesota, Nebraska, North Dakota, Oregon, South Dakota, and Wisconsin (Colosi and Schaal 1997). Introduction as a contaminant in seeds from

Eastern Europe or Asia is the presumed origin of the weedy type in the United States (Bought el al. 1986). Nowadays, wild proso millet is considered as an important weed in corn, sugarbeet, wheat, and soybean (WSSA 1992, Wilson 1992).

Successful early colonizers (often weeds) grow rapidly or large, develop all stages of their life cycles, and eventually are replaced in the environment (Radosevich et al. 1997). Studies of biological characteristics are essential to evaluate the ability of a plant to compete. One of the characteristics that increase competitive ability in a plant community is early and rapid growth (Larcher 1995). Although proso millet is vigorous weed, its biology is not understood.

Proso millet seeds vary in terms of the percent of total seed weight (seed coat) that may be related to seed color (Khan et al. 1996). Proso millet germination can be intermittent over a prolonged time or be quasi-simultaneous (Moore and Cavers 1985). Germination rate has been associated with external and internal factors such as water, oxygen, temperature, and seed size (Raven et al. 1992). In many cases, seed coat impermeability or inhibitory substances can also inhibited germination (Raven et al. 1992, Larcher 1995).

Plant growth analysis, began in the 1920's (Backman 1919, West et al. 1920) and has been used to quantify patterns of dry matter production and leaf area expansion in plants (Radosevich et al. 1997). Plant growth analysis is divided into two distinct approaches: classical and functional (Evans 1972, Causton and Venus 1981, Hunt 1982, Poorter 1989, Hunt 1990), it has been used to compare plant species (Spitters and Kramer 1986, Dunan and Zimdahl 1991, Alcocer-Ruthling et al. 1992, Wall 1993, 1995, Christoffoleti et al. 1997). The classical approach, one of the oldest methods in growth analysis, uses primary data (usually shoot dry weight or leaf area) to calculate relative growth over time (Poorter 1989). The functional method derives growth of plants over time with a mathematical function. Reduction of inter-harvest variability is the main advantage to using fitted curves (Venus and Causton 1979). Richard's function, derived from von Bertalanffy's growth function (Richards 1959), is based on a biologically realistic model as an alternative to polynomial exponential curves for growth analysis (Venus and Causton 1979). Hunt (1982) enumerated several advantages of the functional approach; convenient summary of a complex process and the minimal risk obtained at each harvest. The Richards function has been fitted to evaluate environmental influences on plant growth (Lieth and Reynolds 1986, von Fircks and Verwijst 1993, Lim et al. 1998). For a review of the Richards function's see Hunt (1982).

The objective of this study was to compare growth characteristics and germination of three domestic and nine wild proso millet biotypes from the seed collection of the Colorado State University Weed Science Laboratory.

MATERIALS AND METHODS

Greenhouse studies were performed using nine wild and three domestic proso millet (*Panicum miliaceum* L.) biotypes (Table 2.1.) obtained from the seed collection of the Weed Science Laboratory at Colorado State University. The greenhouse experiments were conducted under 16 hour-day-length supplemented with 1,000-watt metal halide lamps. The temperature in the greenhouse was maintained at 21 - 24°C during the day and 18 - 21°C at night.

Growth analysis

Seeds were germinated at room temperature in plastic boxes on wetted blotting paper. Three germinated seeds of proso millet were transplanted to polyester plastic pots. Each pot (24cm diameter by 25cm depth) was filled with Sunshine® potting mix #1 (Sun Gro Horticulture, Inc., 15831 N.E. 8th Street, Bellevue, Washington 98008). Plants were thinned to one plant per pot. Each pot was considered as an experimental unit (replication). Pots were fertilized with 1.2 g of 15-30-15 Miracle-Gro® (Scotts Miracle-Gro Products, Inc., P.O. Box 888, Port Washington, New York 11050) every 2 weeks.

Five destructive harvests were taken at 14 day intervals starting 14 days after planting (DAP). Harvest periods were 14, 28,42, 56, and 70 DAP. Plants were cut at soil level and dissected into stems, leaves, and panicles. Roots were harvested separately and cleaned in a sieve with running water. Plant height, number of tillers, leaf area, leaf dry weight, stem dry weight, root dry weight, and panicle dry weight waere recorded. Dry weight was obtained by placing fresh materials into paper bags and drying at 65°C for 72 h. Leaf area was determined, before drying, with a leaf area meter (Licor model 3100. Li-cor, Inc., Lincoln , NE, 68583).

The experiment was arranged as a randomized complete block design with four replications. The experiment was performed during May, June and July 1997. The experiment was repeated in 1998 in the same months as in 1997. Richards function was fitted to leaf area and total shoot dry weight. The equation was:

$$\text{Ln}(\mathbf{y}) = \ln(\mathbf{A}) - (1/\boldsymbol{n})*\ln(1 + \exp(\boldsymbol{b} - \boldsymbol{k}*\boldsymbol{DAP})) \qquad (2.1)$$

where **y** is the observation (shoot dry weight or leaf area), the dependent variable, DAP (time expressed in days after planting) is the independent variable, **A** is the asymptotic maximum size of the plant, ***n*** defines the shape of the curve, ***b*** has no biological significance but it positions the curve with respect to the time axis, and ***k*** is a measure of the rate at which **A** is approached (Causton et al. 1978, Garret et al. 1989).

RGR and AGR were derived from the Richards function according to Hunt (1990). The inflexion point was determined following the formulae:

$$\text{Inflexion point (IP)} = (\boldsymbol{k}*\boldsymbol{DAP_0}) - \ln(\boldsymbol{n}) / \boldsymbol{k} \qquad (2.2)$$

where $\boldsymbol{DAP_0}$ is the starting time of plant growth.

Maximum (MaAR) and minimum (MiAR) acceleration ratio was calculated following the formulae reported by Nath and More III (1992):

$$MaAR = IP - (-1/k)*\ln(1/2)*((n + 3)-SQR(n^2 + 6*n +5)). \quad (2.3)$$

$$MiAR = IP + (-1/k)*\ln(1/2)*((n + 3)-SQR(n^2 + 6*n +5)). \quad (2.4)$$

The exponential growth or grand period of growth was estimated by the interval between MaAR and MiAr. Means comparison for IP, MaAR, MiAR, and EG were done by standard error of mean.

Leaf area ratio (LAR), Specific leaf area, and leaf (LWR), stem (SWR), Root (RWR) and reproductive (ReWR) weight ratios expressed as a percent were determined using the following formulae:

$$LAR = L_A/W \ (cm^2\ g^{-1}) \quad (2.5)$$

$$SLA = L_A/L \ (cm^2\ g^{-1}) \quad (2.6)$$

$$LWR = L/W \ (g\ g^{-1}) \quad (2.7)$$

$$SWR = S/W \ (g\ g^{-1}) \quad (2.8)$$

$$RWR = R/W \ (g\ g^{-1}) \quad (2.9)$$

$$ReWR = Re/W \ (g\ g^{-1}) \quad (2.10)$$

where, L_A is the leaf area, and L, S, R, Re, and W are dry weights of leaves, stems, roots, panicles, and plant total dry weight, respectively. The second degree polynomial and an exponential curve were selected to evaluate biomass partitioning and LAR and SLA.

Laboratory experiments

Biotypes were grown in the greenhouse to obtain seeds to be evaluated in the experiments. Forty days after seed harvest, seed weight, seed coat as a percentage of total seed mass, seed color, germination, and root length were determined. Seeds were germinated at room temperature (26 ± 2°C) in plastic boxes on wetted blotting paper. Each box contained 10 seeds was replicated 10 times. Percent of germination was measured 5 and 14 days after incubation (DAI) by visual evaluation, and radicle length 5 DAI. Radicle length was measured from 50 seeds. The experiment was arranged in a completely randomized block design. Seed coat as a percent of total seed mass was evaluated according to Khan et al. (1996) using 10 seeds. Seed weight was measured using 10 replicates of 100 seeds. Each 100 seeds were weighted and the average weight per seed was calculated. Seed color was determined using a Chroma Meter model CR 310 (Minolta) set on the Hunter L (lightness), a (redness), b (yellowness) value scale. The equipment was standardized with a white square which had a pattern L= 97.79, a= 0.022 and b = 1.59. Euclidian distance (ΔE) was calculated using lightness, redness, and yellowness values.

Color difference was expressed in percent and was estimated as follows:

$$\Delta E = \text{SQRT } (\Delta L^2 + \Delta a^2 + \Delta b^2) \qquad (2.11)$$

where ΔE is the square root of square of ΔL, Δa, and Δb, which are the differences between the standard values and the sample values.

Early growth experiment

Five seeds of proso millet were planted one cm deep in Sunshine® potting mix #1 (Sun Gro Horticulture, Inc., 15831 N.E. 8th Street, Bellevue, Washington 98008). Cones measuring 8 cm diameter by 30 cm long (Stuewe and Sons, Inc., Corvallis, OR 97333) were used. After emergence plants were thinned to one plant per cone. Plants grew for 14 days, equal to 18 days after planting (DAP).

Fourteen destructive harvests were taken each day starting 5 DAP. Plants were washed and dissected into roots, leaves and stems. Roots were carefully cleaned in a sieve with running water. Plant heights, leaf area, number of tillers per plant and leaf, stem, and root dry weights were recorded. Dry weight was obtained by placing fresh materials into paper bags and drying at 65°C for 72 h. Leaf area, determined before drying, was determined with a leaf area meter (Licor model 3100. Li-cor, Inc., Lincon, NE, 68583). The experiment was begun in June 1999 using completely randomized block design with 3 replications. Experiment was repeated once in the same summer.

A logarithmic curve was fitted to leaf area, shoot dry weight, and height raw data. The equation was:

$$\ln(y) = y_0 = a * \ln(DAP) \qquad (2.12)$$

where y_0 is the value at DAP = 0 (initial value of parameter analyzed), a is a constant and DAP is time (days after planting).

Data analysis

Data were analyzed using the SAS programming software (SAS institute 1989). Bartlett's test for homogeneity of experimental variances over time was performed (Steel et al. 1997). Mean values for germination, seed coat percent, seed weight, radicle length, lightness, redness, yellowness, and number of tillers per plant were compared using Fisher's protected LSD (P = 0.05). Regression analysis was conducted using the SPSS statistical program (SigmaPlot 4.0 for Windows, SPSS Inc., Chicago, IL, 660611).

RESULTS AND DISCUSSION

Bartlett's test of all data from both studies indicated that experiment variances were not different and that experiments could be combined (Steel et al. 1997).

Growth analysis

Shoot dry weight and leaf area showed significant differences among biotypes. Results of fitting Richards function to shoot dry weight and leaf area combined data are presented in Figures 2.1 to 2.12 and 2.13 to 2.24, respectively. The same figures present the estimated values, RGR and AGR for shoot dry weight or leaf area.

Shoot dry weight

The coefficient of determination for Richards function fitted to shoot dry weight ranged from 0.97 to 0.99. Shoot dry weight over time was estimated using the Richards function. Comparison of curves was done by confidence intervals at 95% (not shown). Canada-Rosemount black seeded with Minnesota-Cambridge olive seeded and South Dakota brown seeded biotypes were the only biotypes that differed at all harvests (Figures 2.1, 2.2 and 2.9). In general, except to Oregon-Grand Island olive seeded and Colorado-Weld County black seeded biotypes, the first period of harvest (14 DAP), all biotypes had a similar growth trend. Ontario, Canada-Huron County black seeded (0.17 g) differed from Wyoming-Platte County brown seeded (0.10 g), Colorado-Weld County black seeded (0.09 g) and Colorado orange seeded biotypes (0.11 g). Colorado-Weld County black seeded differed from Minnesota-Cambridge olive seeded (0.14 g) and South Dakota brown seeded (0.14g). At 26 and 42 days the only differences observed were among Canada-Rosemount black seeded with Minnesota-Cambridge olive seeded and South Dakota brown seeded biotypes.

At 56 and 70 days after planting, differences in growth were observed among biotypes. Maximum accumulation of shoot dry weight, represented by the asymptotic value of A in the Richards function, was obtained by Colorado Weld County tan seeded biotype (26.31 g) after 70 DAP. The lowest shoot dry weight, 13.11 g, was achieved by Canada-Rosemount black seeded biotype at the same harvest period.

The **n** parameter in the Richards function indicates whether or not the regression has an inflection point and where it occurs (Hunt 1982). The inflection point defines where the curve changes from concave to convex (Garret et al. 1989) finishing the self-accelerating phase and starting the self-inhibiting phase (France and Thornley 1984, Nath et al. 1992, Nath et el 1993). A higher **n** means, which ranges from -1 to infinity, a higher inflection point and a greater proportion of the growth curve is exponential. Growth is exponential when the response increases with the increase of AGR, both at about the same rate (Nath et al. 1992). Inflection points calculated from the Richards function for all biotypes are presented in Table 2.2. Oregon and Wyoming biotypes showed the highest percent of exponential growth phase for the total growth period while the lowest was to Canada-Rosemount. The inflection points ranged from 18 to 25 days. The inflexion point for domestic and wild biotypes averaged 21 and 23 days, respectively.

The grand period of growth lies between the extremes of the absolute acceleration rate (Nath et al 1992, Nath et al 1993). This period approximates a straight line cumulative growth (constant rate) where maximum and minimum acceleration rate (MaAR and MiAR, respectively), are the points which its slope is zero (Nath et el. 1992). The grand period of growth for proso millet biotypes estimated by shoot dry weight varied from 6 to 17 days (Table 2.2). Canada-Rosemount and South Dakota were the biotypes with the shortest grand period of growth, 6 and 9 days respectively, while Wyoming had the longest (17 days): however, not different to Minesota-Cambridge and Colorado-Weld County tan seeded.

Figures 2.1 to 2.12 show relative and absolute growth rates of shoot dry weight, derived from the Richards function. Mean RGR and AGR with 95% confidence interval calculated from the Richards function are reported in Table 2.3. Although no differences were seen in RGR, shoot AGR differed among biotypes. The average RGR for all 12 biotypes was 0.095 g g^{-1} day^{-1}. Colorado tan seeded shoot AGR differed from Wyoming, Colorado orange seeded, Colorado black seeded and Canada-Rosemount biotypes. Canada-Rosemount, the lowest shoot AGR also showed differences from 5 other biotypes. Although no significant differences were observed in some shoot AGR between domestic and wild, wild biotypes tended to have greater shoot AGR than domestic biotypes. Average AGR for domestic proso milled biotypes was 0.31 ± 0.02 g day^{-1} while the average for the wild types was 0.36 ± 0.02 g day^{-1}.

Leaf area

The determination coefficient for leaf area from the fit of the Richards function varied from 0.92 to 0.96 (Figures 2.13 to 2.24). The Richards function did not detected differences among biotype leaf area prior to 26 DAP. This result implies that the biotypes had the same photosynthetic area or the same surfaces to intercept light and produce energy. Canada-Rosemount showed significant differences of leaf area to the tan seeded and Oregon biotypes at 42 DAP.

A similar tendency was observed 56 and 70 DAP. Olive and tan seeded biotypes had the highest leaf area expansion differing from two black seeded wild biotype, Canada-Rosemount and Colorado, and one domestic biotypes, Colorado orange seeded. The average leaf area 70 DAP, for the three biotypes with the lowest leaf area expansion, was 621 cm corresponding to 54% of the average of olive and tan seeded leaf area expansion.

The grand period of growth for leaf area among biotypes was between 17 and 26 days (Table 2.2.). Even though Wyoming biotype had the longest value of leaf area growth grand period, it was not significant different from biotypes that showed values higher than 22 days. The estimated leaf area inflection points varied from 33 to 37 days.

Graphs of estimated leaf area RGR and AGR are showed in Figures 2.13 to 2.24 and mean values are presented in Table 2.3. Leaf area RGR was not significantly different among biotypes averaging 0.062 $cm^2\ cm^{-2}day^{-1}$. Canada-Rosemount and Colorado black seeded have the lowest average increment of leaf area a day, estimated in 11 $cm^2\ day^{-1}$. This increment was statistically different from the increase of 22 $cm^2\ day^{-1}$ observed for the Colorado tan seeded biotype. Domestic and wild biotypes averaged leaf AGR 14 $cm^2\ day^{-1}$ and 19 $cm^2\ day^{-1}$, respectively. Because of the smaller expansion leaf area observed for domestic biotypes, wild biotypes might be more able to compete for space than domestic biotypes. This may imply a greater competitive ability for wild biotypes. This was expected due to adaptation in different environments observed for this weed.

Leaf area ratio (LAR) and Specific leaf area (SLA)

The destructive harvest 70 DAP was not included in the analysis of LAR and SLA caused by the difficulty in washing and cleaning the root system to include in total dry weight by biotypes. LAR and SLA are represented in Figure 2.25. Biotypes did not show significant differences in Leaf Area Ratio. The initial parameter (y_0) for the fitted nonlinear equation was not significant in some regression analysis; however, the parameter was maintained for both LAR and SLA due to fitting the same equation for the biotypes. LAR mean value was 310 cm^2g^{-1} and 51 cm^2g^{-1} 14 and 56 DAP, respectively. Colorado-Larimer biotype had the highest SLA, 387 cm^2g^{-1}. Although Canada-Rosemount had the lowest RGR and AGR, it had one of the highest SLA among the biotypes studied, 337 cm^2g^{-1}.

Plant height and tiller number

Proso millet biotype height over time was evaluated by fitting a nonlinear logarithmic regression (Figures 2.26 to 2.37). The analysis of variance showed differences for the interaction of biotype and harvest date as well for individual factors. According to Bought et al. (1986) usually proso millet plants range from 30 to 100 cm tall but are frequently up to 150 cm when they grow in crops such as corn (Bought and Cavers 1987). The estimated average plant height of domestic and wild proso millet was 130 and 145 cm, respectively, after 70 days.

The only differences verified up to 26 days were between Colorado-Larimer, the taller, with two black seeded, Canada-Rosemount and Colorado, and one domestic, Colorado white seeded, biotypes. The ability of a weed to grow faster can imply the ability to compete for the available resources. At 42, 56 and 70 DAP similar responses was observed. All biotypes, 70 DAP, followed the same trend observed 42 DAP. Colorado-Larimer did not differ in plant height from Oregon, Ontario Canada-Huron County and Colorado tan seeded biotypes. The shorter biotype, Colorado orange seeded, grew just 122 cm, which was not significant different from Wyoming, Colorado black seeded and Colorado white seeded biotypes.

Even though all biotypes developed 2 to 4 tillers, significant differences ($P < 0.098$) were observed among Nebraska (2.1 tillers/plant) and Minnesota-Cambridge (3.4 tillers/plant), Canada-Rosemount (3.1 tillers/plant), Oregon (3.2 tillers/plant), and Ontario, Canada-Huron County (3.5 tillers/plant) biotypes.

Biomass accumulation

Biomass accumulation analysis was done using four destructive harvests. A second degree polynomial was selected to fit all four parts of the plant: leaves, stems, roots, and reproductive tissue (panicle including seed when present) (Figure 2.38 to 2.49). During the first 2 weeks after planting, 60% of total plant biomass was allocated to photosynthetic tissue. At the same time, 23 and 17% was partitioned to stem and root dry weight, respectively. Wall (1995) compared three cruciferous weeds using biomass partitioning. In his results, 70% of total biomass was allocated to photosynthetic tissues during the first 2 weeks after emergence.

Although the regression fitted to root dry weight was significant, the regression coefficient was not greater than 0.21. Proso millet biotypes allocated not more than 22% to root dry weight. The average biomass proportion allocated to root tissue for the biotypes was 16%.

The inversion point, where stem contributes more to total dry weight than leaves, occurred between 25 to 35 days for all biotypes. Colorado black seeded changed the proportion of biomass at 27 days while Wyoming did so at 34 days. Minnesota, Oregon, Colorado-Larimer, and the three domestic, biotypes started to develop reproductive tissues in the third week after planting. For the other biotypes, reproductive tissues started one week late. Cardenas et al (1983) described three phenological stages of proso millet:

vegetative, reproductive and ripening. The vegetative phase is defined as the period from germination to panicle initiation. Usually, this phase is completed 16 to 20 days after planting; however, it was extended in our experiments for Nebraska-Panhandle Center tan seeded and for Wyoming-Platte County brown seeded biotypes to around 38 days (Figure 2.42 and 2.43).

Reproductive tissue differed at 56 DAP among biotypes. Estimated by the regression curves, South Dakota, Colorado orange seeded, Canada-Rosemount, Colorado black seeded, and Colorado Larimer biotypes allocated 29, 27, 25, 23, 20% of total biomass to panicle production. The black seeded biotypes presented different patterns of allocation over time. Ontario had smaller panicle production than the other two black seeded biotypes. Its panicle proportion of total biomass was 9% while for the others the average was 24% at 56 DAP. The results demonstrate different growth characteristics for biotypes independent of seed color. With our results we can propose that proso millet biotypes may have different competitive ability because of differences observed in growth parameters evaluated.

Laboratory experiments

Seed color

In 1931, the Commission Internationale de L'Éclairage[1] developed a system for specifying color stimuli using tristimulus values. It was modified in 1976, and is now called CIE 1976 colour space or CIELAB, the system uses a three dimensional color space known as **L** (lightness), **a** (redness-greenness) and **b** (yellowness-blueness). **L** value ranges from 0 (black) to 100 (white) (Westland)[2]. Lightness, redness, yellowness, and Euclidean distance (ΔE) of the proso millet studied are presented in Table 2.4.

Lightness of proso millet seed ranged from 32.35 to 47.63. Although the white seeded biotypes showed the highest **L** values, Colorado domestic was significantly different from the Colorado wild biotype. The third lightest biotype was Colorado orange, which differed from all other biotypes. The olive, tan, and brown seeded biotypes ranged in lightness from 33.73 to 35.52, except South Dakota brown seeded that showed the lowest **L** value

[1] Commission Internationale de L'Éclairage, an organization devoted to international cooperation and exchange of information among its member countries on all matters relating to the science and art of lighting, http://www.hike.te.chiba-u.ac.jp/ikeda/CIE/home.html

[2]. Frequently asked questions about colour physics prepared by Stephen Westland at Colourware, http://www.colourware.co.uk/cpfaq.htm

measured, 32.35, not different to the black biotypes (32.48 to 33.03).

Positive, negative and zero **a** values indicate red, blue and gray color, respectively, interpreted as redness. Redness of the biotypes varied from 1.61 to 7.67. Colorado orange, the third reddest, was statistically different from all other biotypes. The white seeded Colorado wild biotype showed a different **a** value than Colorado domestic white seeded biotype. Even though differences were observed among the **a** values of black seeded biotypes, these showed a narrow redness interval with values varying from 2.29 to 2.81. Minnesota-Cambridge olive had the lowest redness value, which was statistically different from Oregon olive.

Positive, negative, and zero **b** values measure yellow, green, and gray color, respectively, and are interpreted as yellowness. The **b** values ranged from 1.14 to 13.11. White and orange seeded biotypes had the highest **b** values while the yellowness for the black seeded and South Dakota brown seeded biotypes did not exceed 1.74. Except for South Dakota, the other biotypes with tan, brown and olive seeds had **b** values between 2.58 and 4.56.

South Dakota brown seeded biotypes had the highest overall color distance (ΔE); however, no differences were observed for Canada-Rosemount and Colorado black seeded biotypes. In the description of the major weedy biotypes of proso millet in Canada reported by Bought et al. (1986), black, bronze, olive-green, or dark brown were classified among the black biotypes. The white and orange seeded biotypes showed to be the closest biotypes to the white pattern but significant differences among them were detected.

Khan et al. (1996) reported similar results for seed color of eleven proso millet populations, measured by maximum light absorption at 400 and 500nm. Seed color ranged from 0.124 to 0.339 where white, and golden yellow seeded biotypes had the lowest values and the dark red and black seeded biotypes had the highest.

Seed coat percent

Seed coat as a percent of total seed mass are presented in Table 2.5. Seed coat ranged from 12.2 to 29.8%. Khan et al. (1996) in similar study with proso millet biotypes reported that the seed coat ranged from 12.6 to 24.5%. Even though differences were not observed between the olive seeded, tan seeded, Canada-Rosemount and Ontario, Canada black seeded, and Wyoming brown seeded biotype, olive seeded biotypes tended to have greater value of seed coat averaged in 29.1%. Khan et al. (1996) observed that black seeded biotypes had a higher % of total weight as seed coat than all other biotypes analyzed. Black seed coat varied from 20.2 to 26.3. The black seeded biotypes from Canada showed higher percent of seed coat than the black seeded biotype from Colorado. The domestic and white seeded wild biotype biotypes had seed coat percent between 12.2 and 18.8%, similar to the range reported by Khan et al. (1996).

Seed weight

Seed weight ranged from 5.3 to 7.5 mg (Table 2.5). Bough et al. (1986) states that seed weight of proso millet ranged from 3.0 to 6.6 mg per seed depending on the biotype. Mean weight per seed changes in different seasons (Kane and Cavers 1991). Proso millet seeds produced early in October declined 1.2 mg per seed when compared to seeds produced in the middle of August. Our results showed that South Dakota and Colorado domestic white biotype showed the heaviest seed weights. Black seeded biotypes did not differ in seed weight. Black seed weight averaged 5.6 mg $seed^{-1}$ which was higher than the average observed early in the season (2 mg) and late in the season (3.0 mg) (Bough et al. 1996).

Germination and radicle length

Although Bough et al. (1996) suggested that the germination of eleven proso millet biotypes achieved maximum germination from 36 to 108 hours. Seeds from different origin in our results showed germination rate less than 28% 14 DAI (Table 2.5). According to Bough et al. (1986), black pigmented seeds took the longest period to reach maximum germination. In this study, Ontario, Canada-Huron County black seeded germinated 91% after 5 days while Canada-Rosemount and Colorado-Weld County black seeded did not achieve more than 28% germination 14 DAI. Fresh seeds of black proso millet are dormant, unlike the other weedy biotypes (Bough et al 1986). Also they stated that dark red fresh seed germination rate was 15% with wide differences among replicates. In previous experiments conducted by Kane and Cavers (1991), fresh black seeds of proso millet germination ranged between 5 and 20%.

Tan seed biotypes as well the Wyoming biotype also germinated less than 28% 14 DAI. The white, orange, and olive biotypes had germination rates greater than 98% 14

DAI. This result agrees with the germination rate reported by Bough et al. (1996)

Radicle length measured 5 DAI varied from 0.2 to 5.1 cm (Figure 2.50). Biotypes that showed the highest germination rate 5 DAI, had the longest radicle length. Domestic proso millet biotypes produced radicles faster than wild biotypes. The two black seeded biotypes with low germination rates were among the biotypes with the shortest radicle length.

Early growth experiment

Plant height

Plots of height vs time are shown in Figures 2.51 to 2.62. Confidence intervals estimated were used to compare early growth curves (data not shown). Colorado domestic white and orange seeded biotypes grew faster than Colorado black seeded and Oregon olive seeded biotypes throughout the experimental period. Also the Colorado domestic white seeded biotype was taller than the Nebraska biotype. The highest predicted plant height, 14 DAP, was achieved by Colorado domestic white seeded (15.3 ± 1.1 cm); however, no significant differences were observed with, South Dakota and Colorado orange seeded biotype. Colorado white seeded biotype previously reached 20.9 ± 7.0 cm 14 DAP), but no differences were detected up to 26 DAP among biotypes. Differences could be detected in this experiment due to one day harvest interval, this reduced the variation detected previously. Domestic biotypes had no different plant height over the experimental period averaging 22.2 cm tall 18 DAP, except for the period between 7 and 13 DAP for South Dakota that was different form domestic biotypes from Colorado. Canada-Rosemount was the shortest biotypes, even though no differences were detected for the Colorado black seeded.

Brown seeded and tan seeded biotypes among the seeded color group biotypes plant height was not different throughout 18 DAP while other groups showed differences over time. Olive seeded were different from the first harvest period (5 DAP). As described before, two black biotypes had no significant differences for plant heights but differences were detected with Ontario biotype.

Leaf area

Leaf area analysis detected significant differences for the interaction of biotype and harvest; therefore plots of leaf area vs time were built (Figures 2.63 to 2.74). Differences among biotypes ($P < 0.0001$) were observed. Both Colorado domestic biotypes did not show significant differences in leaf area expansion throughout the experiment. Ontario black seeded leaf area was not different to Colorado black seeded biotype throughout the experiment. Differences between Canada-Rosemount and the other two black seeded biotypes were observed 9 and 13 DAP. Olive seeded biotypes had different leaf area expansion between 5 and 15 DAP. Similar results were detected for brown and tan seeded biotypes.

Canada-Rosemount and Oregon olive seeded biotypes had smaller leaf area expansion than all biotypes. Domestic biotypes originated from Colorado showed greater leaf area expansion than the domestic biotype from South Dakota.

Even though the highest leaf area expansion estimated 14 DAP, 26.7 ± 1.2 cm^2, was observed for Colorado domestic white seeded, no differences were detected for both Colorado wild white seeded and Colorado domestic orange seeded biotypes. Besides the recorded value was 31.5 ± 4.8 cm^2. In contrast, leaf area expansion measured 14 and 26 DAP did not differ among biotypes in the growth analysis experiment. In that study, leaf area expansion estimated for Colorado domestic white seeded biotype was 38.10 ± 1.57

cm^2, value predicted 16 DAP in this experiment. The variability observed among harvest period in the previous experiment may explain differences observed between experiments. The narrowest interval among harvests in this study contributed to decrease the previously variance detected. The smallest leaf area expansion, in both experiments 14 DAP, was observed for Canada-Rosemount biotype, even though no significant differences were observed for Oregon-Grand Island olive seeded.

Shoot dry weight

Plots of shoot dry weight vs time are presented in Figures 2.75 to 2.86. Differences of shoot dry weight accumulation were detected among biotypes ($P < 0.0001$).

As for leaf area expansion, similar shoot dry weight curves were detected among biotypes. Minnesota shoot dry weight was not significantly different to Ontario, Colorado tan seeded and Wyoming shoot dry weight. Equivalent trends were also observed between Oregon and Canada-Rosemount, Nebraska, and Colorado black seeded. Although the Wyoming biotype did not accumulate as much shoot dry weight as Colorado black seeded, its shoot dry weight accumulation was not different to Ontario black seeded biotype. Wyoming and Colorado tan seeded increased shoot dry weight in the same proportion throughout the experiment. South Dakota accumulated the same shoot dry weight as Colorado-Larimer biotype.

Domestic biotypes differed throughout the harvest period from both black seeded biotypes, Canada-Rosemount and Colorado-Weld County, and Oregon-Grand Island olive seeded biotype; however, no differences were detected 18 DAP for the Ontario biotype and the South Dakota biotype. Nebraska biotype accumulated less shoot dry weight than both Colorado domestic biotypes, the same occurred with Wyoming and Colorado tan seeded biotypes. Wyoming was also different from Colorado-Larimer biotype.

The two domestic Colorado biotypes accumulated 0.23 g of shoot dry weight after 18 DAP, this value was higher than other biotypes, except for Colorado-Larimer. The lowest shoot dry weight (0.09 g) was observed for Canada-Rosemount, even though no differences were detected between it and Oregon.

Previous experiments estimated shoot dry weight variation between 0.09 and 0.17 g plant^{-1} 14 DAP, while the variation in this study ranged from 0.04 to 0.10 g plant^{-1}. To reach those values in this experiment, proso millet biotypes had to grow 18 days. The differences detected in the average was due to the higher variation observed in the previous experiment than this experiment

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Biomass accumulation

Leaf (LWR), root (RWR), and shoot (SWR) ratios evaluated 11 and 18 DAP, equivalent to 7 and 14 days after emergence, are presented in Table 2.6. Comparison of ratio means was done using the standard error of the mean. LWR ranged from 45 to 64% and 46 and 53% 11 and 18 DAP, respectively. Even though, biotypes with brown, tan, and white seed color did not show significant differences in LWR 11 DAP, differences were detected 18 DAP, except for the white biotypes. Differences among black seeded biotypes as well between olive seeded biotypes were detected in both evaluations. Colorado domestic biotypes had the same LWR; even though, the orange seeded differed from South Dakota brown seeded 11 DAP.

RWR ranged from 19 to 32% and 22 to 36% 11 and 18 DAP, respectively. In general, RWR was maintained after 18 days, even though some change was verified. Colorado-Larimer increased 12% root weight proportion from 11 to 18 DAP. A similar trend was verified for the Minnesota biotype, with an increasing of around 10%.

SWR varied from 10 to 18% and between 18 and 24% 11 and 18 DAP, respectively. Although the lowest SWR was verified for Canada-Rosemount, similarity was detected to other biotypes.

Plant partitioning observed in these experiment emphasize differences observed in proso millet biotypes in the field. Color seed is an important aspect to distinguish biotypes but growth characteristics also are important. Based on the results wild proso millet may have different competitive ability due to different growth patterns. It is expected that biotypes with greater leaf area expansion and greater dry weight accumulation compete more with other species due to the needs (nutrient, water, space).

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Table 2.1. Origin, panicle architecture and seed color of proso millet (*Panicum miliaceum* L.) biotypes used in the growth analysis studies.

ACCESSION NUMBER	**ORIGIN**	**TYPE**	**PANICLE ARCHITECTURE**	**SEED COLOR**
1	Minnesota -Cambridge	Wild	Open	Olive
4	Canada-Rosemount	Wild	Open	Black
8	Oregon-Grand Island	Wild	Open	Olive
9	Ontario, Canada-Huron County	Wild	Open	Black
10	Nebraska-Panhandle Center	Wild	Open	Tan
12	Wyoming-Platte County	Wild	Dense, Droop	Brown
13	Colorado-Weld County	Wild	Dense, Droop	Black
15	Colorado-Weld County	Wild	Open	Tan
16	South Dakota	Domestic	Open	Brown
17	Colorado	Domestic	Dense	White
18	Colorado	Domestic	Dense	Orange
21	Colorado-Larimer	Wild	Semi-open	white

Table 2.2. Inflection point (IP), maximum and minimum acceleration rate (MaAR and MiAR, respectively) expressed in days after planting, estimated from the fitted Richards function parameters for proso millet biotypes.

BIOTYPE	TYPE	SHOOT DRY WEIGHT				LEAF AREA			
		IP	MaAR	MiAR	EG[1]	IP	MaAR	MiAR	EG
Minnesota -Cambridge	wild	23	16	30	14	34	24	44	20
Canada-Rosemount	wild	18	15	21	6	34	24	43	19
Oregon-Grand Island	wild	25	19	31	12	36	25	47	22
Ontario, Canada-Huron County	wild	21	15	27	12	35	23	47	24
Nebraska-Panhandle Center	wild	24	17	30	13	37	26	48	22
Wyoming-Platte County	wild	25	16	33	17	37	24	50	26
Colorado-Weld County	wild	22	16	28	12	33	24	42	18
Colorado-Weld County	wild	24	17	32	15	37	25	49	24
South Dakota	domestic	20	16	25	9	33	25	41	16
Colorado	domestic	23	17	30	13	36	25	47	22
Colorado	domestic	21	16	26	10	34	26	43	17
Colorado-Larimer	wild	22	17	27	10	35	27	44	17
Standard Error of the mean		0.6	0.3	1	1.9	0.4	0.3	0.9	2.1

[1] Exponential growth

Table 2.3. Mean absolute (AGR) and relative (RGR) shoot dry weight growth rate's of proso millet biotypes.

BIOTYPE	TYPE	SHOOT DRY WEIGHT		LEAF AREA	
		AGR[1] $(g\ day^{-1})$	RGR $(g\ g^{-1}\ day^{-1})$	AGR $(cm^2\ day^{-1})$	RGR $(cm^2\ cm^{-2}\ day^{-1})$
Minnesota -Cambridge	wild	0.39^{ABCD}	0.094	20.36^{AB}	0.065
Canada-Rosemount	wild	0.24^{E}	0.092	10.90^{B}	0.065
Oregon-Grand Island	wild	0.42^{AB}	0.098	20.56^{AB}	0.065
Ontario, Canada-Huron County	wild	0.37^{ABCD}	0.090	16.38^{AB}	0.057
Nebraska-Panhandle Center	wild	0.41^{ABC}	0.099	21.16^{AB}	0.069
Wyoming-Platte County	wild	0.31^{CDE}	0.096	14.66^{AB}	0.064
Colorado-Weld County	wild	0.26^{DE}	0.096	10.97^{B}	0.055
Colorado-Weld County	wild	0.45^{A}	0.100	21.91^{AB}	0.066
South Dakota	domestic	0.35^{ABCDE}	0.092	14.00^{AB}	0.057
Colorado	domestic	0.32^{BCDE}	0.095	15.70^{AB}	0.061
Colorado	domestic	0.28^{CDE}	0.092	12.72^{AB}	0.061
Colorado-Larimer	wild	0.38^{ABCD}	0.096	17.57^{AB}	0.061

[1] Means followed by the same superscript in a row are not significantly different by standard error of the mean

2.4. Seed color of domestic and wild proso millet biotypes, as determined by the Hunter lightness (**L**), redness (**a**), yellowness (**b**), and overall color distance (DE)

BIOTYPE	TYPE	SEED COLOR	L	a	b	DE
Minnesota-Cambridge	wild	Olive	35.28	1.61	3.77	62.55
Canada-Rosemount	wild	Black	33.02	2.81	1.74	64.81
Oregon-Grand Island	wild	Olive	33.73	2.15	2.58	64.09
Ontario, Canada-Huron County	wild	Black	32.48	2.29	1.14	63.37
Nebraska-Panhandle Center	wild	Tan	35.52	3.07	4.56	62.34
Wyoming-Platte County	wild	Brown	35.16	3.05	4.11	62.68
Colorado-Weld County	wild	Black	33.03	2.69	1.74	64.81
Colorado-Weld County	wild	Tan	33.96	2.63	3.15	63.89
South Dakota	domestic	Brown	32.35	2.52	1.44	65.98
Colorado	domestic	White	47.63	3.13	13.11	50.67
Colorado	domestic	Orange	39.46	7.66	11.11	59.1
Colorado-Larimer	wild	White	42.93	5.32	12.51	55.01
LSD (0.05)			0.03	0.05	0.04	0.61

Table 2.5. Seed coat as percent of total seed mass, seed weight and seed germination of proso millet biotypes 5 and 14 days after seed incubation.

BIOTYPE	TYPE	SEED COLOR	SEED COAT	SEED WEIGHT (mg)	GERMINATION 7[2]	14
Minnesota-Cambridge	wild	Olive	28.4[1]	6.6	98	98
Canada-Rosemount	wild	Black	26.3	5.5	13	17
Oregon-Grand Island	wild	Olive	29.8	5.3	82	98
Ontario, Canada-Huron County	wild	Black	25.0	5.7	91	91
Nebraska-Panhandle Center	wild	Tan	27.5	6.5	11	28
Wyoming-Platte County	wild	Brown	24.0	6.5	11	18
Colorado-Weld County	wild	Black	20.2	5.5	23	23
Colorado-Weld County	wild	Tan	24.0	6.3	14	28
South Dakota	domestic	Brown	17.8	7.5	98	100
Colorado	domestic	White	12.2	7.5	100	100
Colorado	domestic	Orange	13.8	6.7	100	100
Colorado-Larimer	wild	White	18.8	5.6	100	100
LSD (0.05)			4.0	0.3	1.3	1.1.

[1] Expressed in percent of total seed mass
[2] Days after incubation

Table 2.6. Plant partitioning of total proso millet dry weight: leaf weight ratio (LWR), root weight ratio (RWR), and stem weight ratio (SWR).

BIOTYPE	TYPE	HARVEST					
		7[1]			14		
		LWR	RWR	SWR	LWR	RWR	SWR
Minnesota-Cambridge	wild	62.6[2] ± 2.2	19.2 ± 2.1	18.1 ± 2.4	49.4 ± 0.6	29.3 ± 0.7	21.3 ± 0.6
Canada-Rosemount	wild	64.1 ± 1.3	25.9 ± 1.7	10.0 ± 0.5	46.1 ± 0.8	36.0 ± 1.0	18.0 ± 1.0
Oregon-Grand Island	wild	54.3 ± 2.1	31.9 ± 3.2	13.8 ± 1.5	51.9 ± 1.1	28.6 ± 1.1	19.5 ± 1.1
Ontario, Canada-Huron County	wild	60.4 ± 1.7	22.9 ± 2.4	16.7 ± 1.9	48.3 ± 0.6	31.8 ± 1.1	19.9 ± 0.8
Nebraska-Panhandle Center	wild	58.1 ± 4.0	24.5 ± 3.5	17.5 ± 2.2	53.4 ± 0.9	24.0 ± 1.3	22.6 ± 0.7
Wyoming-Platte County	wild	55.9 ± 2.8	28.6 ± 1.7	15.5 ± 1.5	52.5 ± 1.0	23.9 ± 1.4	23.7 ± 0.7
Colorado-Weld County	wild	56.5 ± 1.4	27.4 ± 2.0	16.1 ± 1.9	50.0 ± 2.4	28.9 ± 2.7	21.2 ± 1.0
Colorado-Weld County	wild	57.8 ± 3.4	26.7 ± 3.5	15.5 ± 2.0	50.8 ± 0.8	27.8 ± 1.1	21.4 ± 0.8
South Dakota	domestic	45.4 ± 9.2	24.6 ± 5.4	13.3 ± 3.2	45.8 ± 1.3	33.0 ± 2.1	21.2 ± 1.1
Colorado	domestic	55.1 ± 1.8	28.9 ± 3.4	16.1 ± 3.2	48.4 ± 2.3	28.5 ± 2.7	23.1 ± 1.3
Colorado	domestic	57.9 ± 1.9	25.5 ± 1.9	16.6 ± 2.3	48.0 ± 1.2	29.0 ± 2.4	23.0 ± 1.3
Colorado-Larimer	wild	59.9 ± 2.9	21.5 ± 1.4	18.2 ± 1.7	46.9 ± 1.2	34.1 ± 2.1	19.0 ± 1.0

1 Days after emergence

2 Data are means ± standard error used to compare biotypes

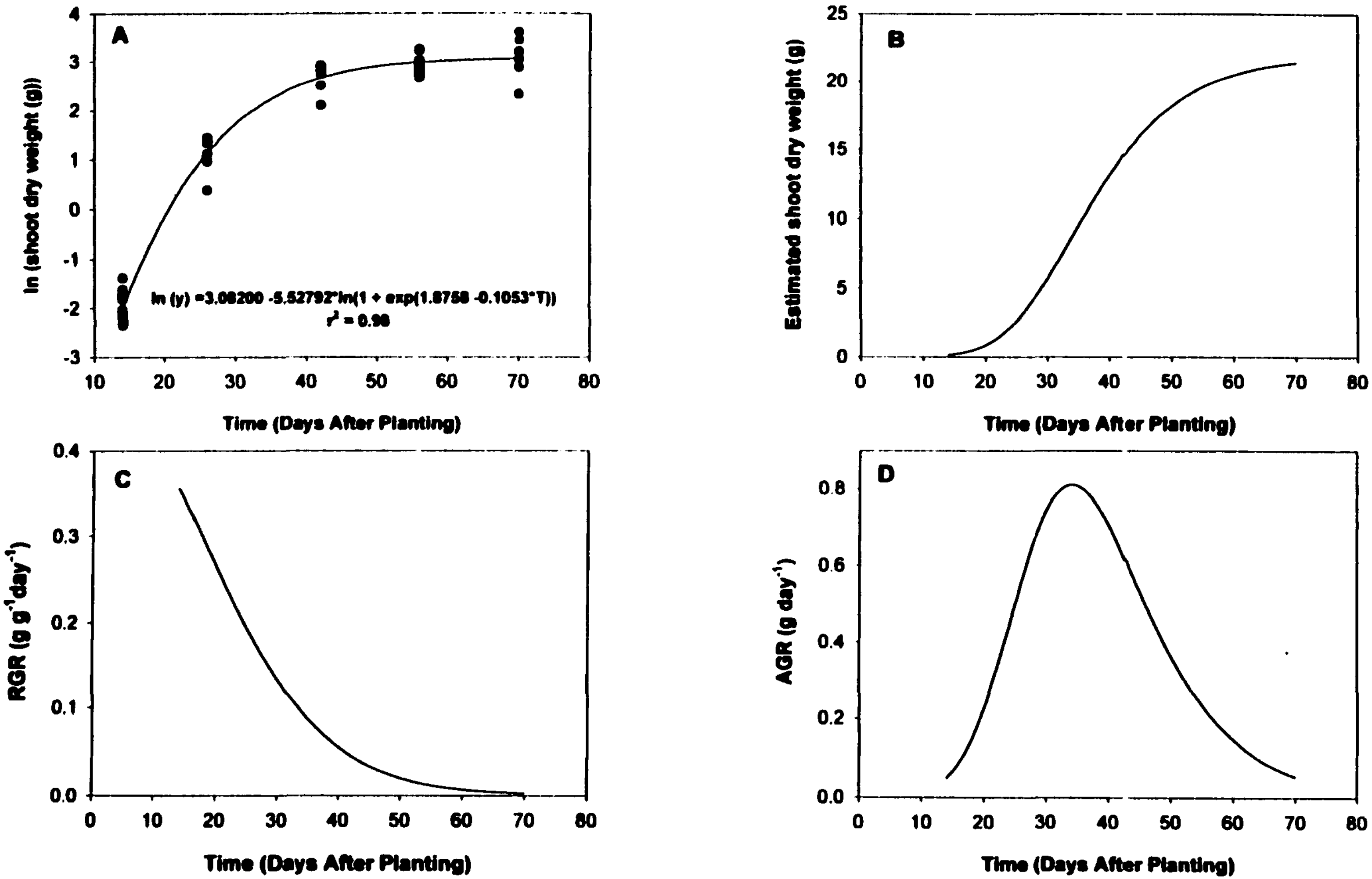

Figure 2.1. Richards function fitted curve (A), estimated shoot dry weight (B), shoot relative growth rate (C), and shoot absolute growth rate (D) of Minnesota-Cambridge olive seeded proso millet biotype.

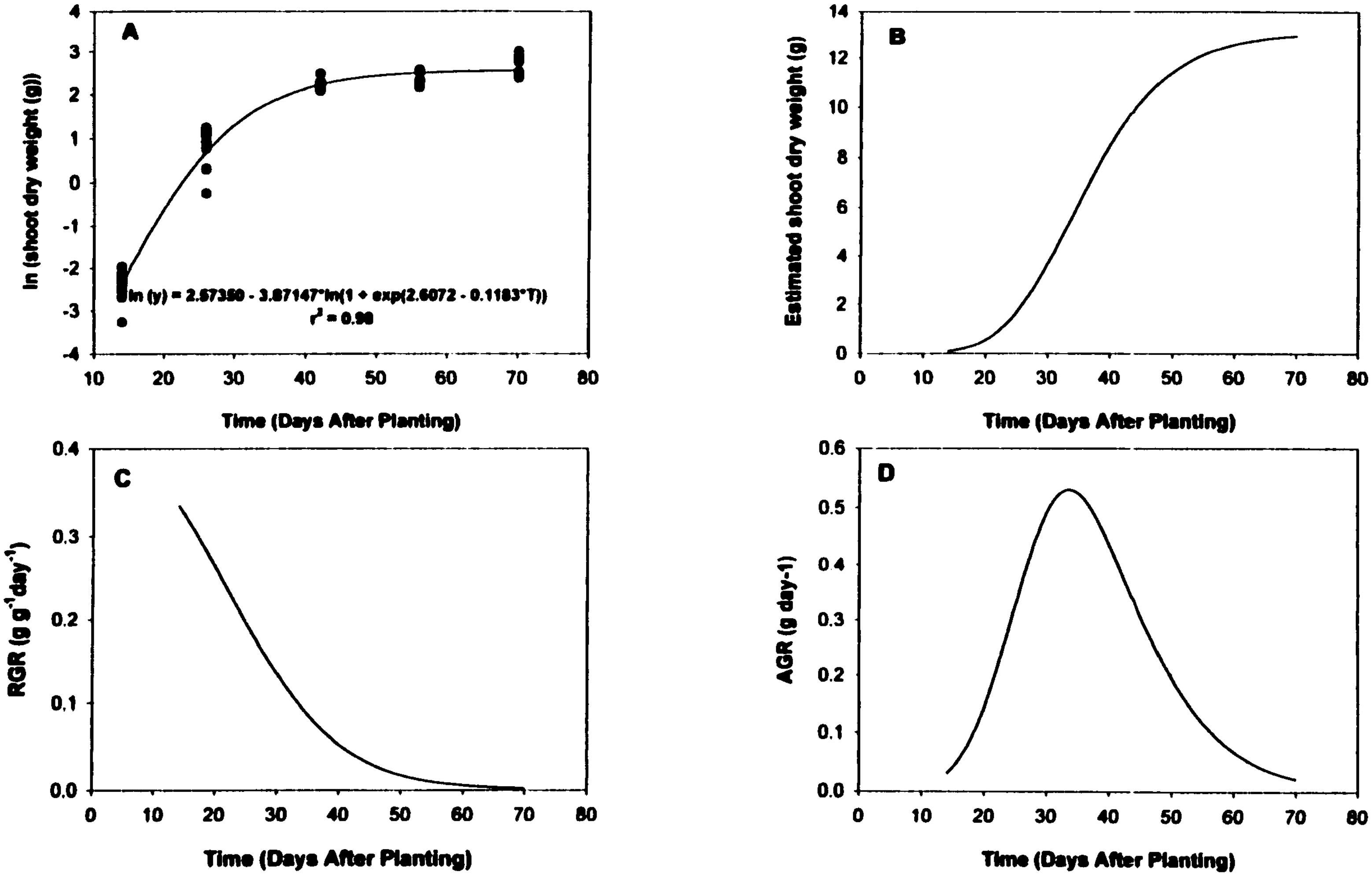

Figure 2.2. Richards function fitted curve (A), estimated shoot dry weight (B), shoot relative growth rate (C), and shoot absolute growth rate (D) of Canada-Rosemount black seeded proso millet biotype.

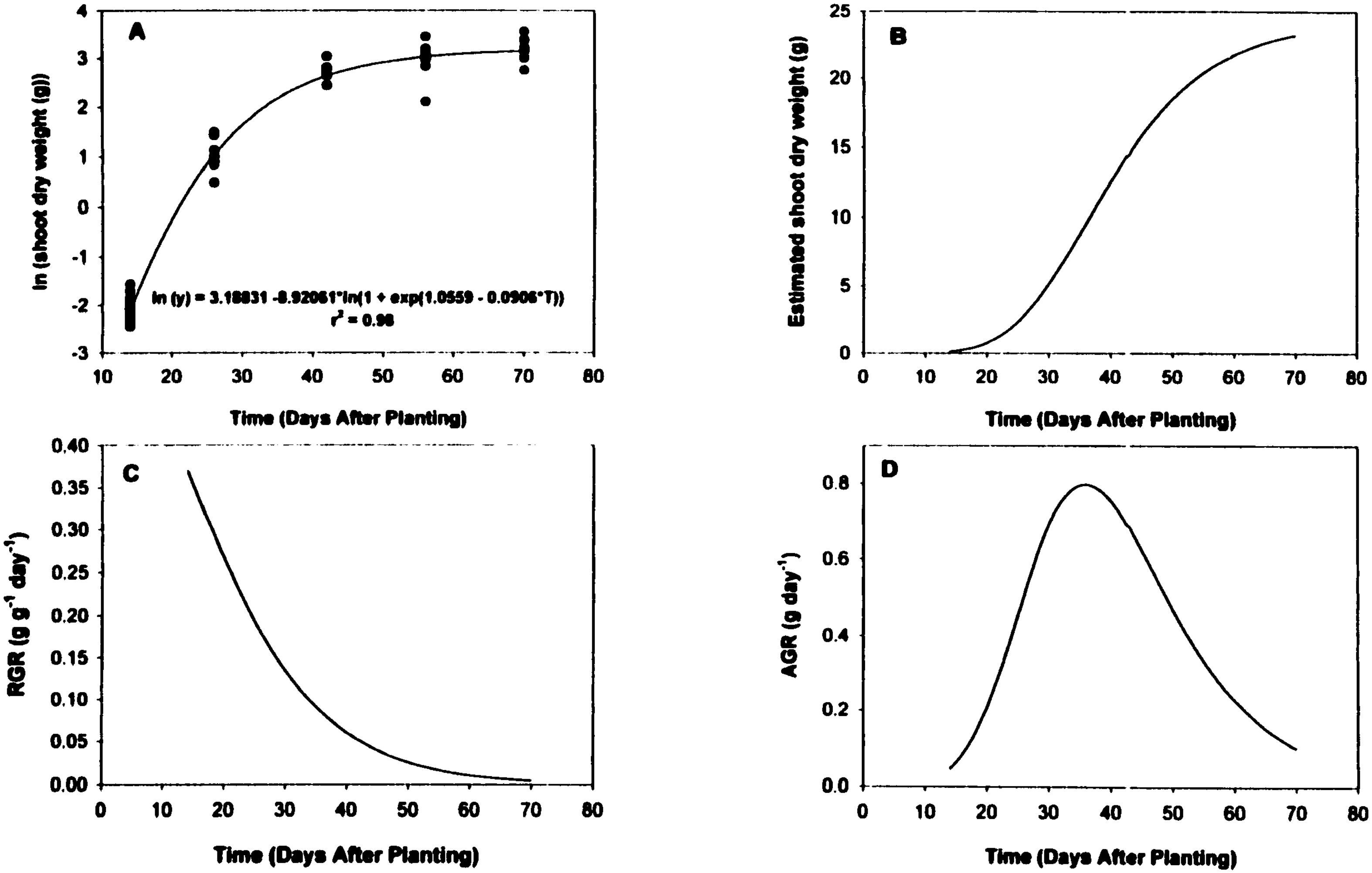

Figure 2.3. Richards function fitted curve (A), estimated shoot dry weight (B), shoot relative growth rate (C), and shoot absolute growth rate (D) of Oregon-Grand Island olive seeded proso millet biotype.

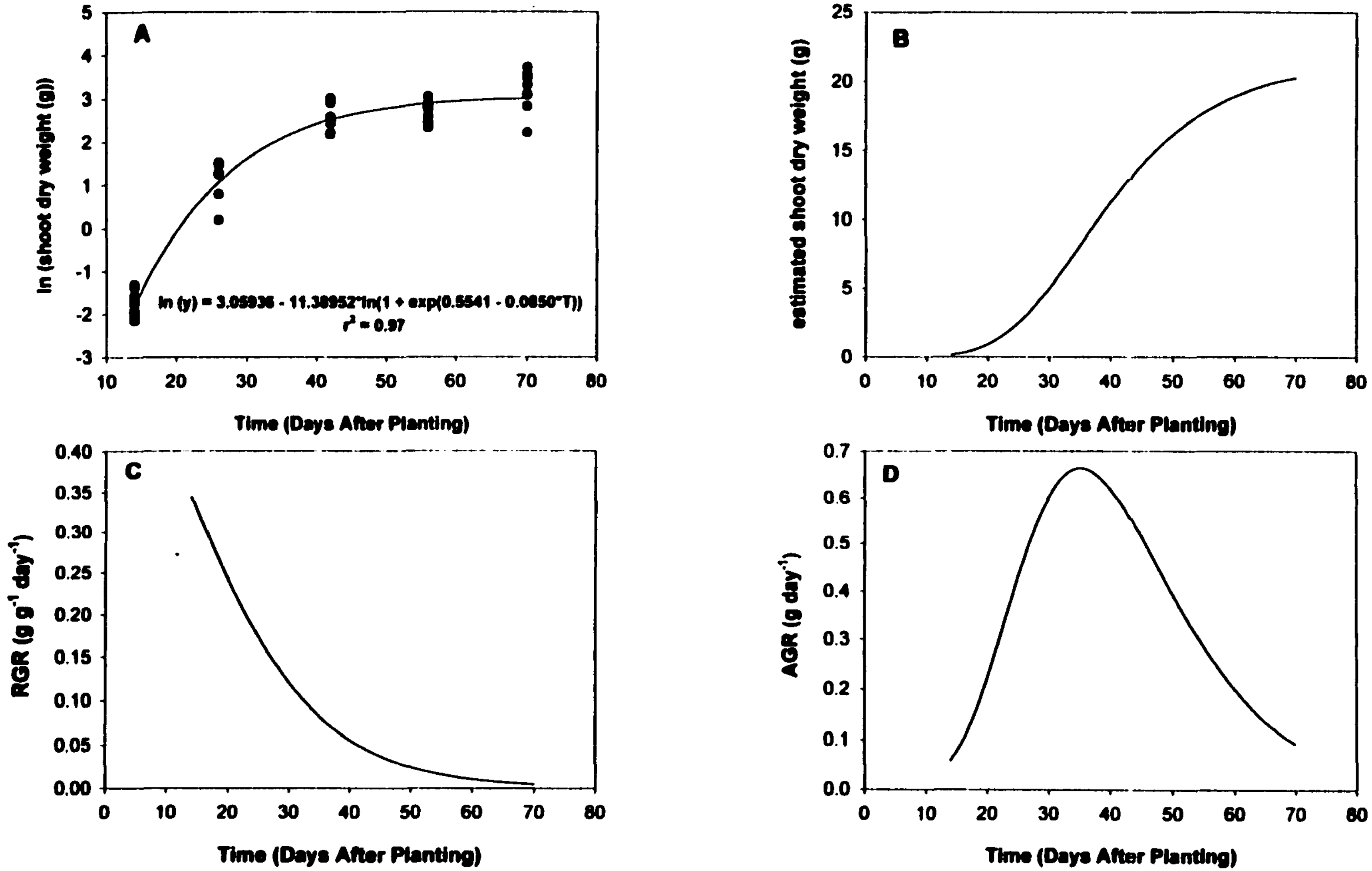

Figure 2.4. Richards function fitted curve (A), estimated shoot dry weight (B), shoot relative growth rate (C), and shoot absolute growth rate (D) of Ontario, Canada-Huron Count black seeded proso millet biotype.

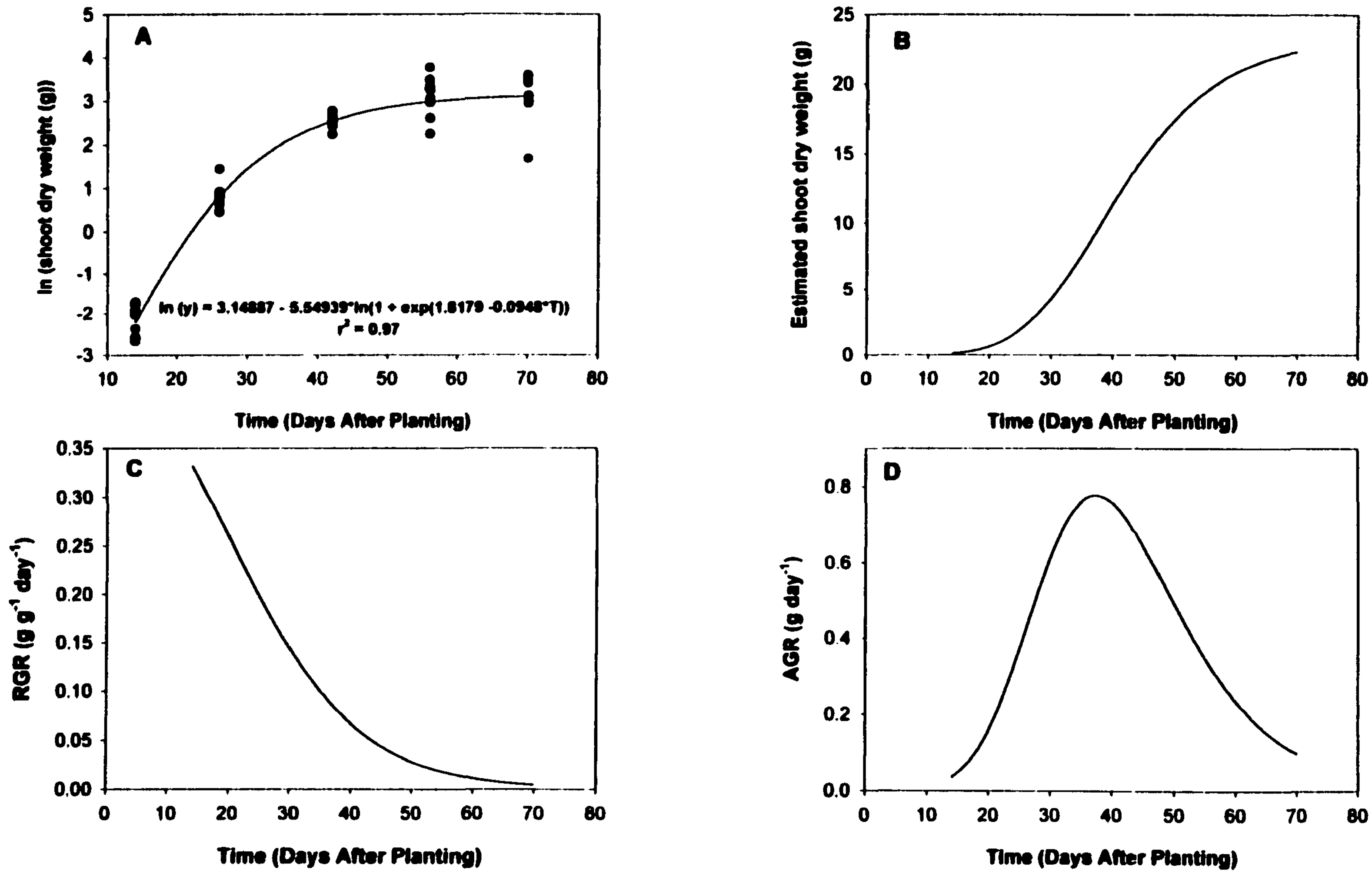

Figure 2.5. Richards function fitted curve (A), estimated shoot dry weight (B), shoot relative growth rate (C), and shoot absolute growth rate (D) of Nebraska-Panhandle center tan seeded proso millet biotype.

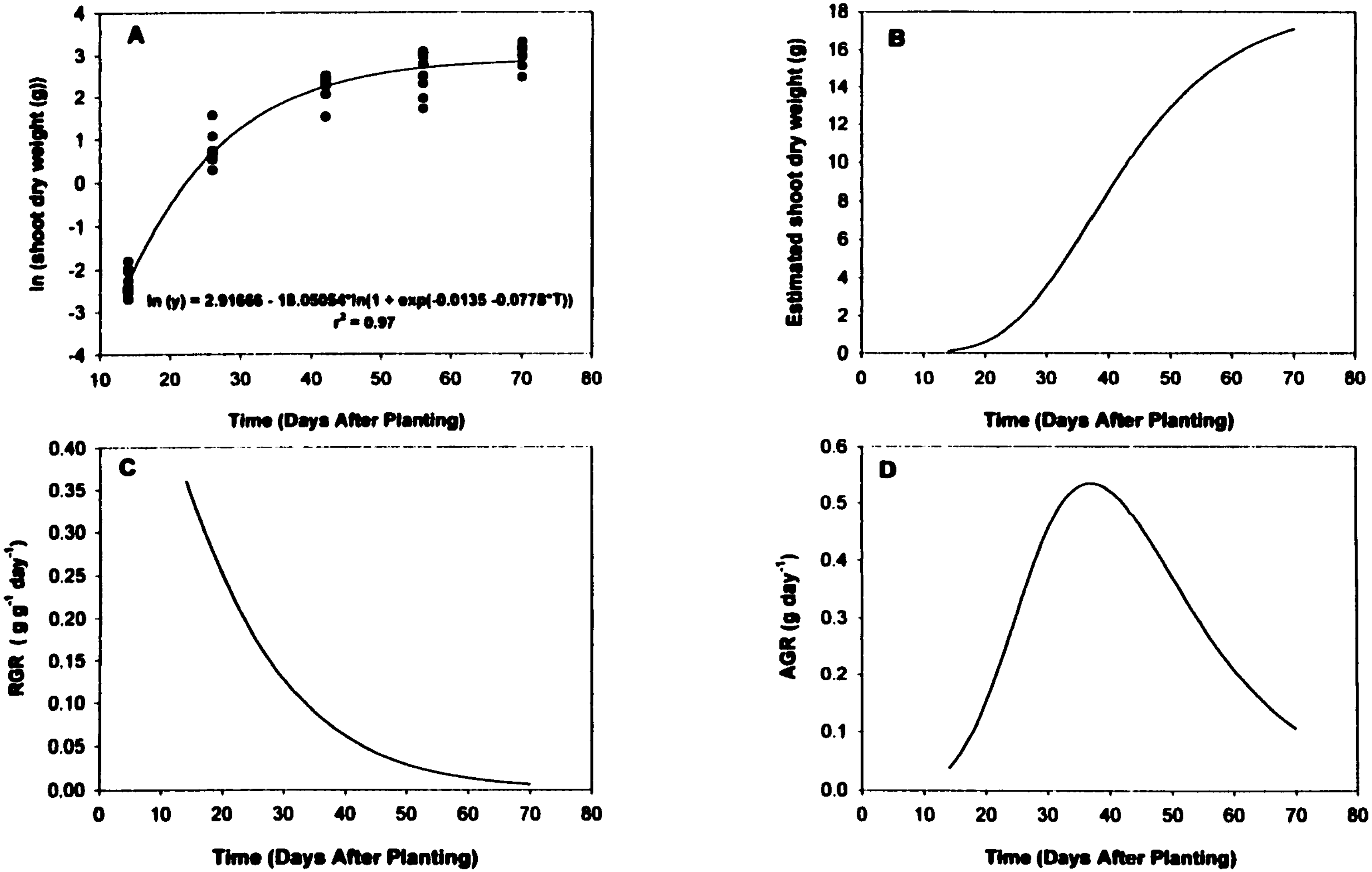

Figure 2.6. Richards function fitted curve (A), estimated shoot dry weight (B), shoot relative growth rate (C), and shoot absolute growth rate (D) of Wyoming-Platte County brown seeded proso millet biotype.

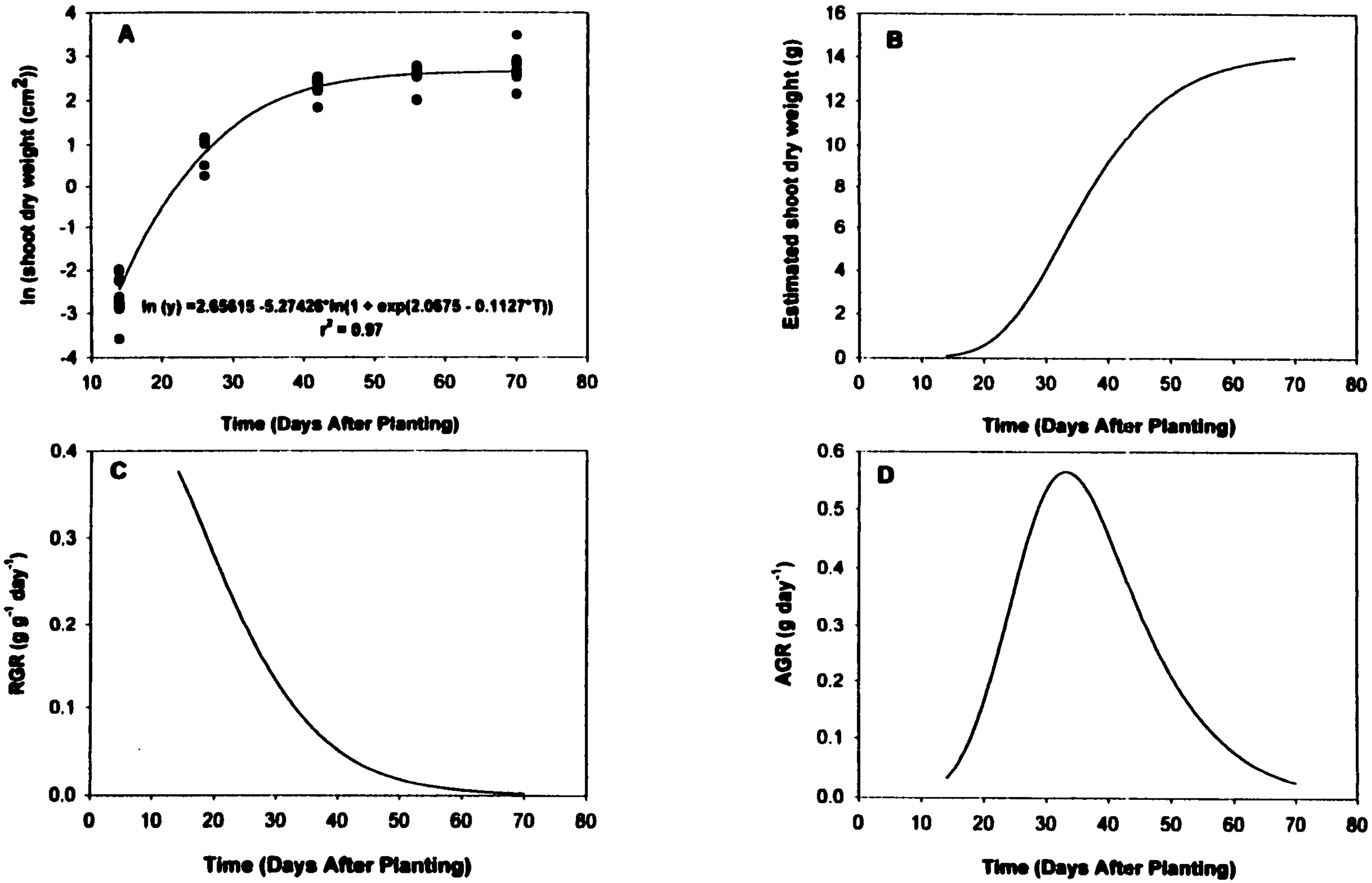

Figure 2.7. Richards function fitted curve (A), estimated shoot dry weight (B), shoot relative growth rate (C), and shoot absolute growth rate (D) of Colorado-Weld county black seeded proso millet biotype.

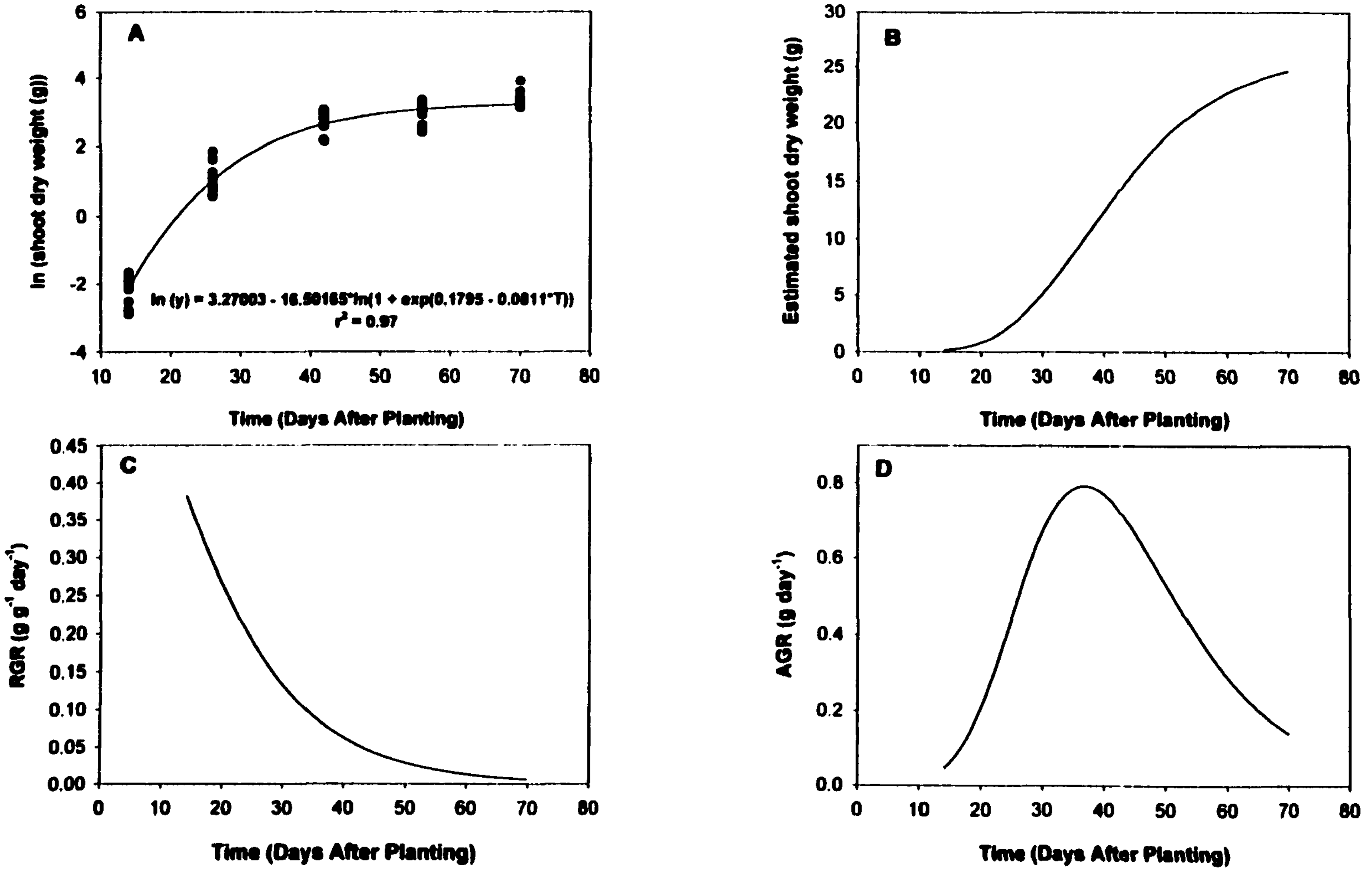

Figure 2.8. Richards function fitted curve (A), estimated shoot dry weight (B), shoot relative growth rate (C), and shoot absolute growth rate (D) of Colorado-Weld County tan seeded proso millet biotype.

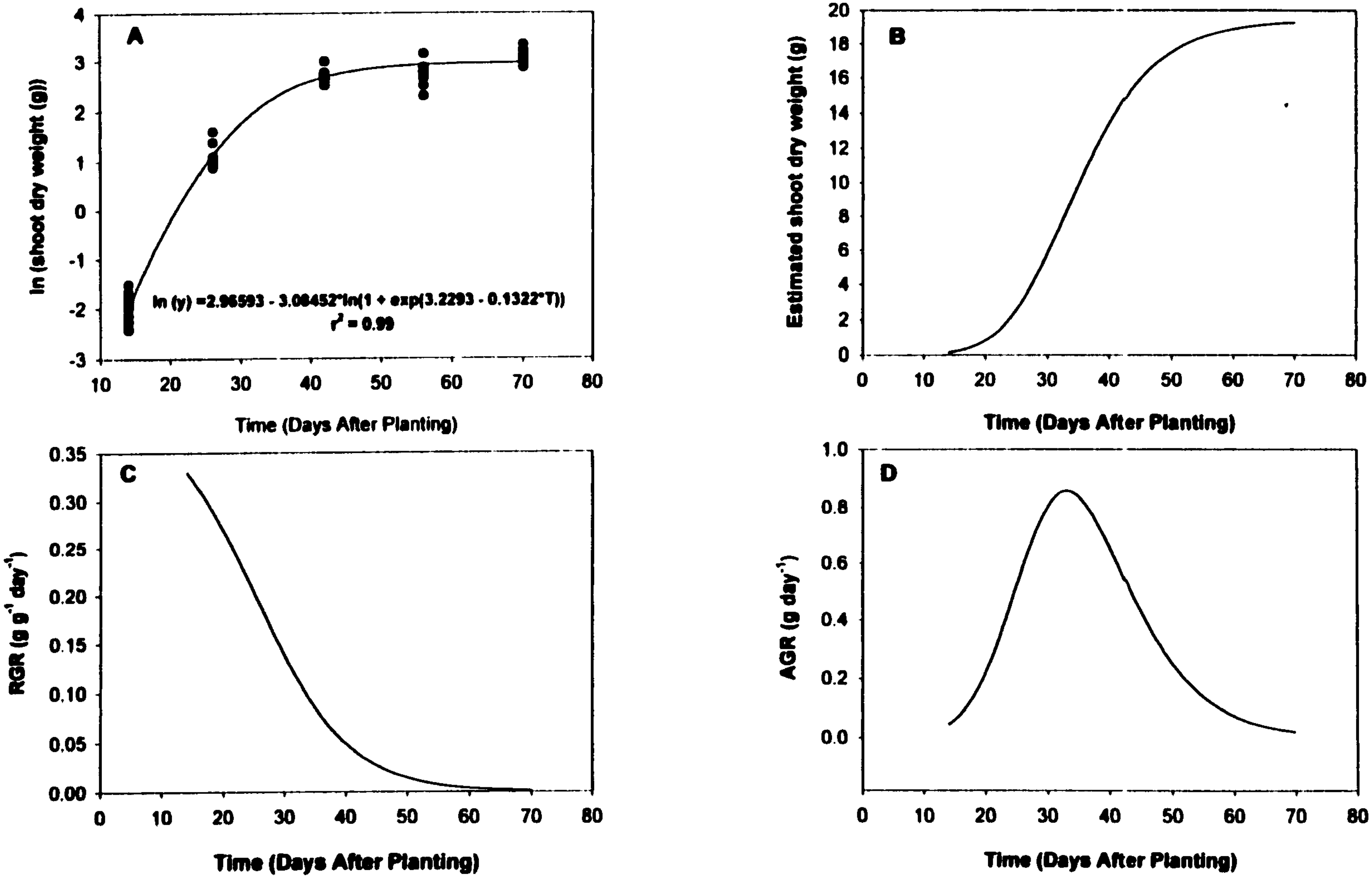

Figure 2.9. Richards function fitted curve (A), estimated shoot dry weight (B), shoot relative growth rate (C), and shoot absolute growth rate (D) of South Dakota brown seeded proso millet biotype.

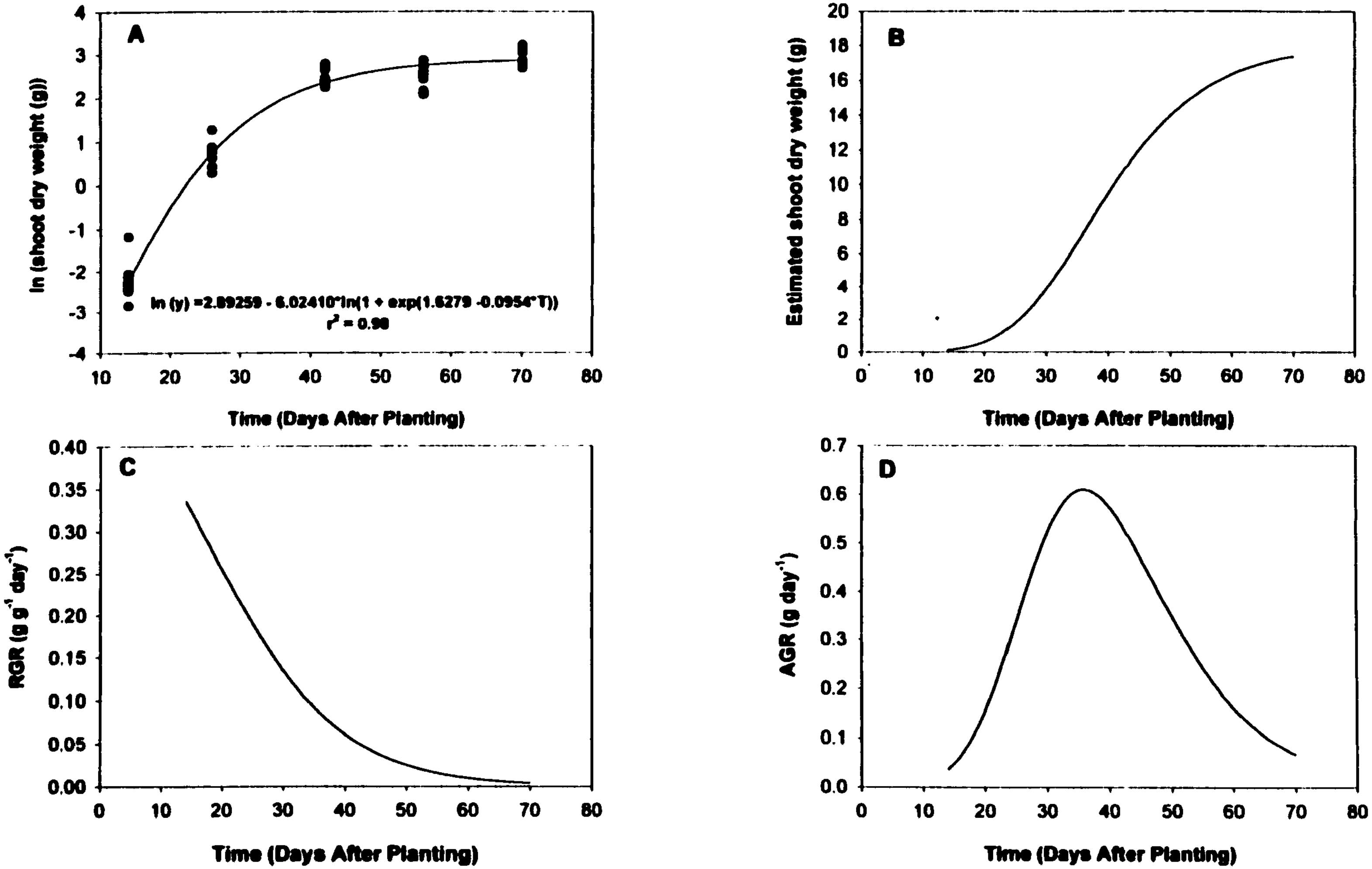

Figure 2.10. Richards function fitted curve (A), estimated shoot dry weight (B), shoot relative growth rate (C), and shoot absolute growth rate (D) of Colorado white seeded proso millet biotype.

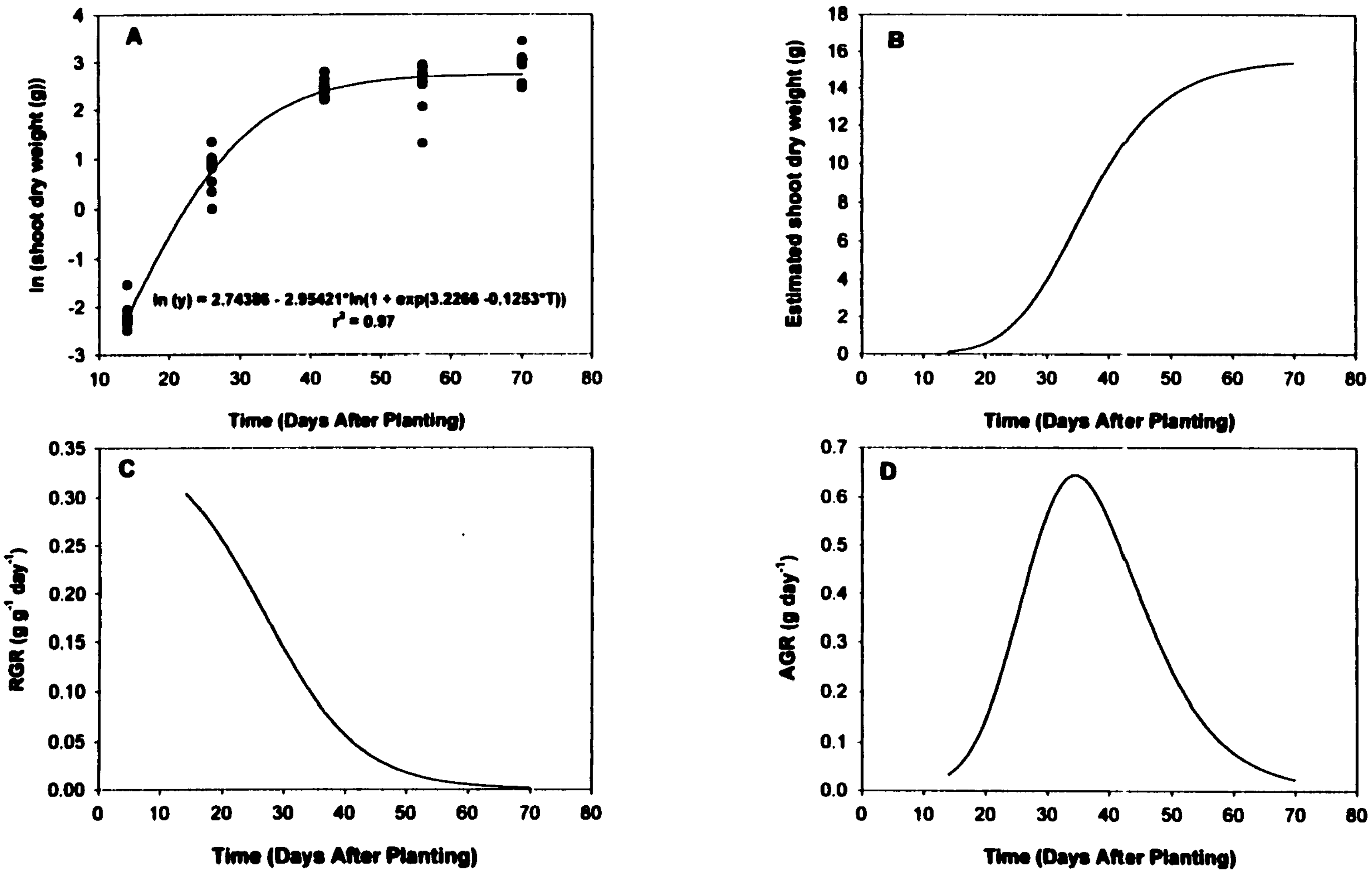

Figure 2.11. Richards function fitted curve (A), estimated shoot dry weight (B), shoot relative growth rate (C), and shoot absolute growth rate (D) of Colorado orange seeded proso millet biotype.

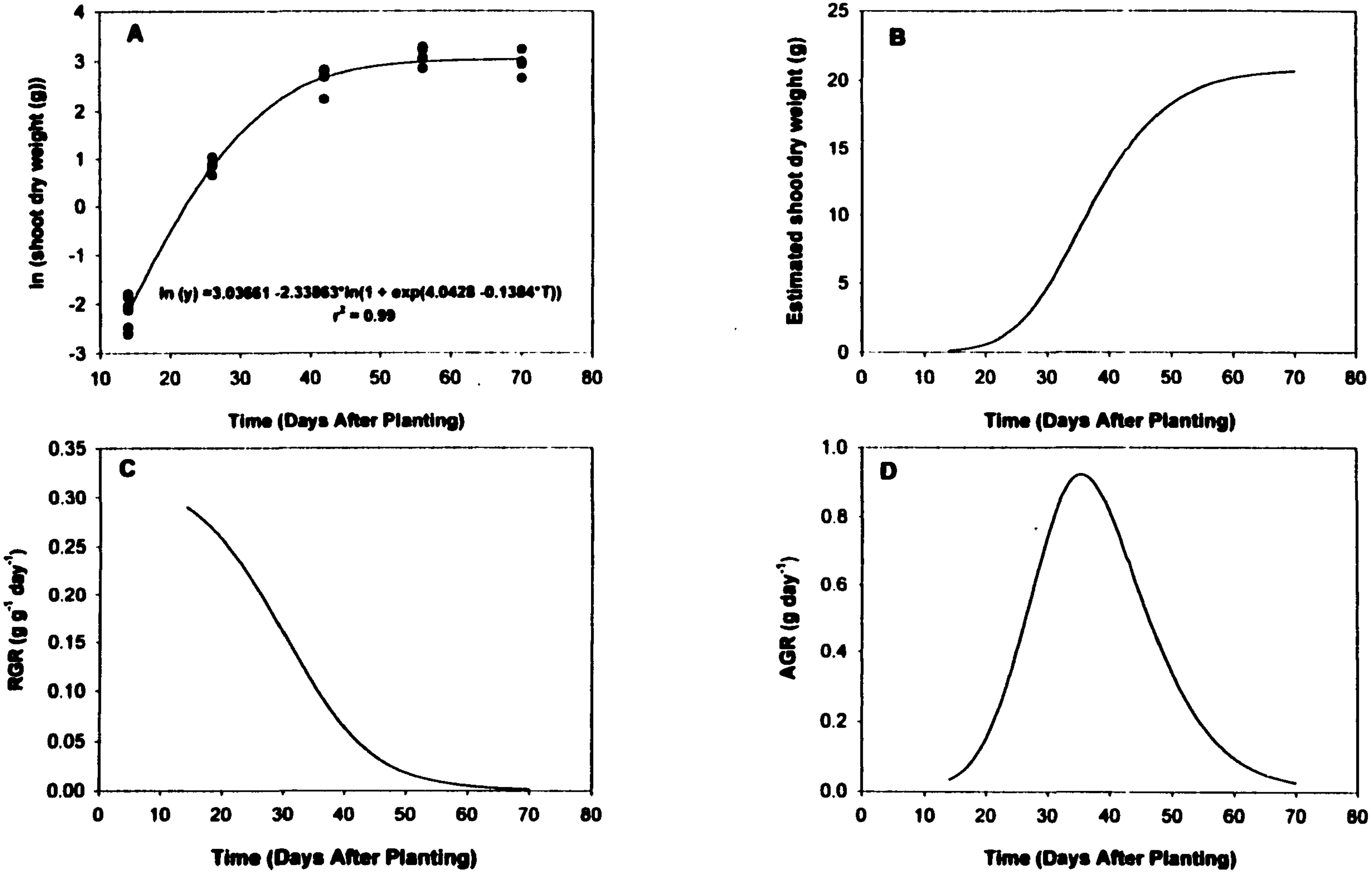

Figure 2.12. Richards function fitted curve (A), estimated shoot dry weight (B), shoot relative growth rate (C), and shoot absolute growth rate (D) of Colorado-Larimer white seeded proso millet biotype.

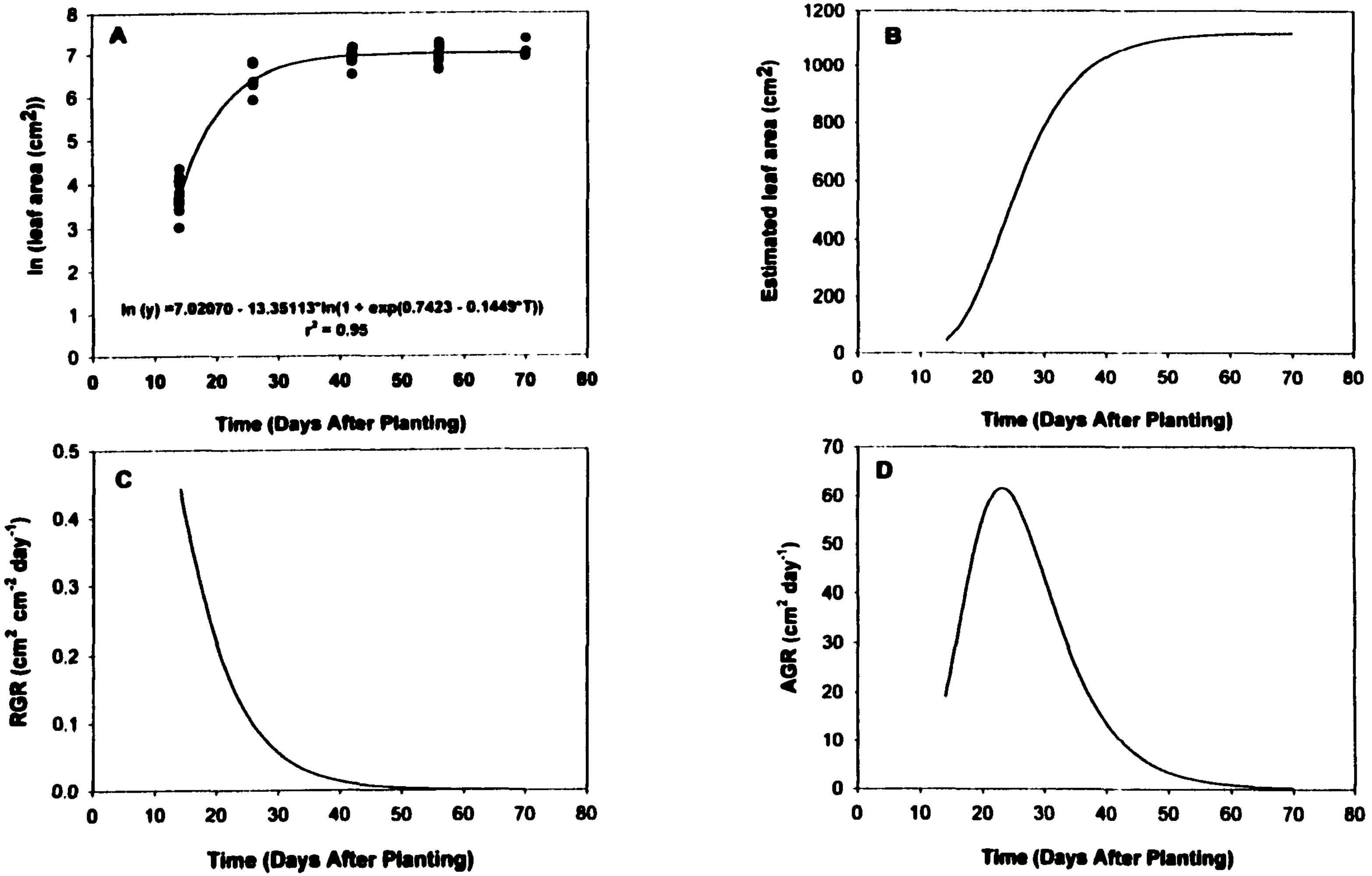

Figure 2.13. Richards function fitted curve (A), estimated leaf area (B), leaf area relative growth rate (C), and leaf area absolute growth rate (D) of Minnesota-Cambridge olive seeded proso millet biotype.

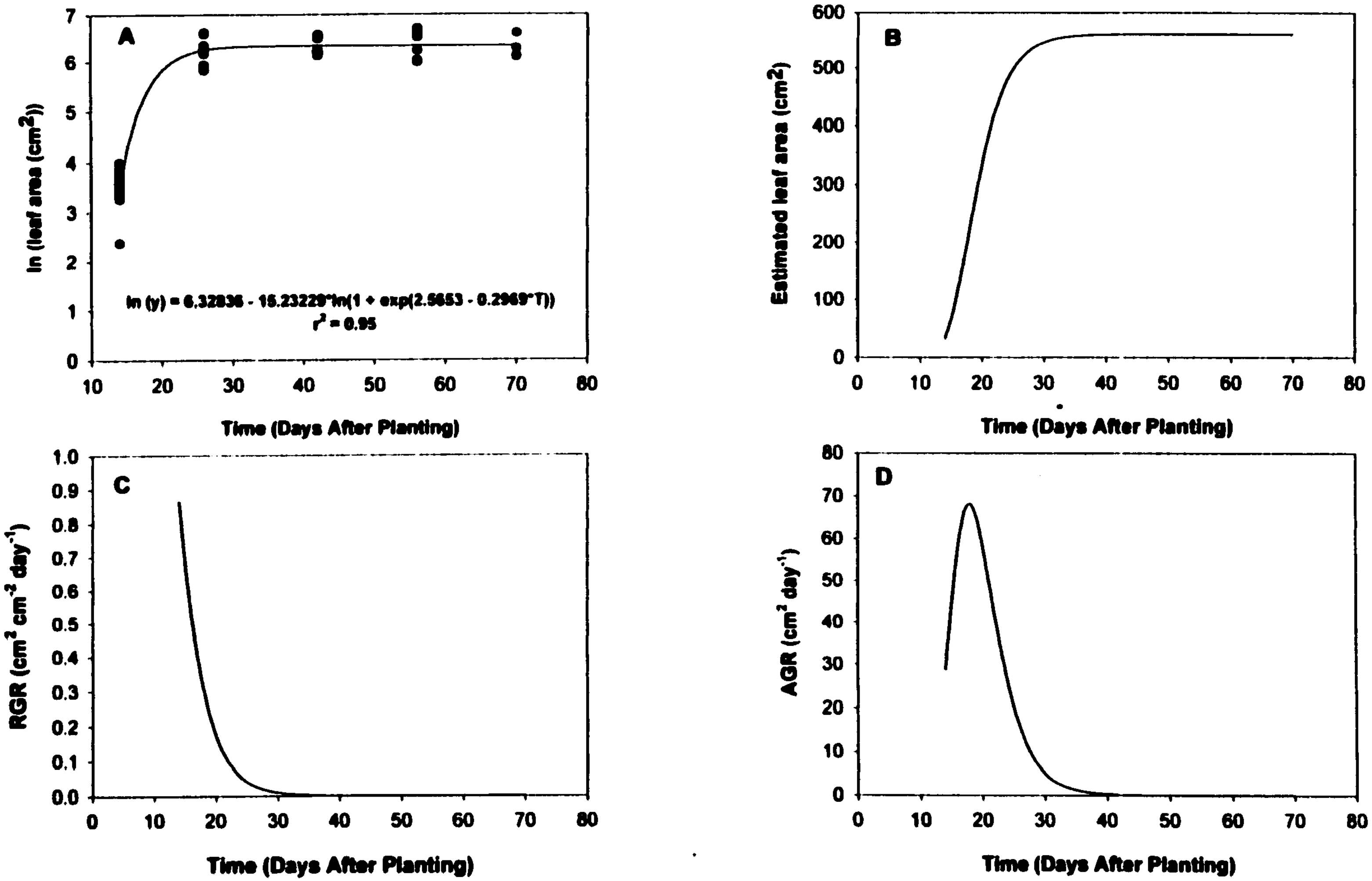

Figure 2.14. Richards function fitted curve (A), estimated leaf area (B), leaf area relative growth rate (C), and leaf area absolute growth rate (D) of Canada-Rosemount black seeded proso millet biotype.

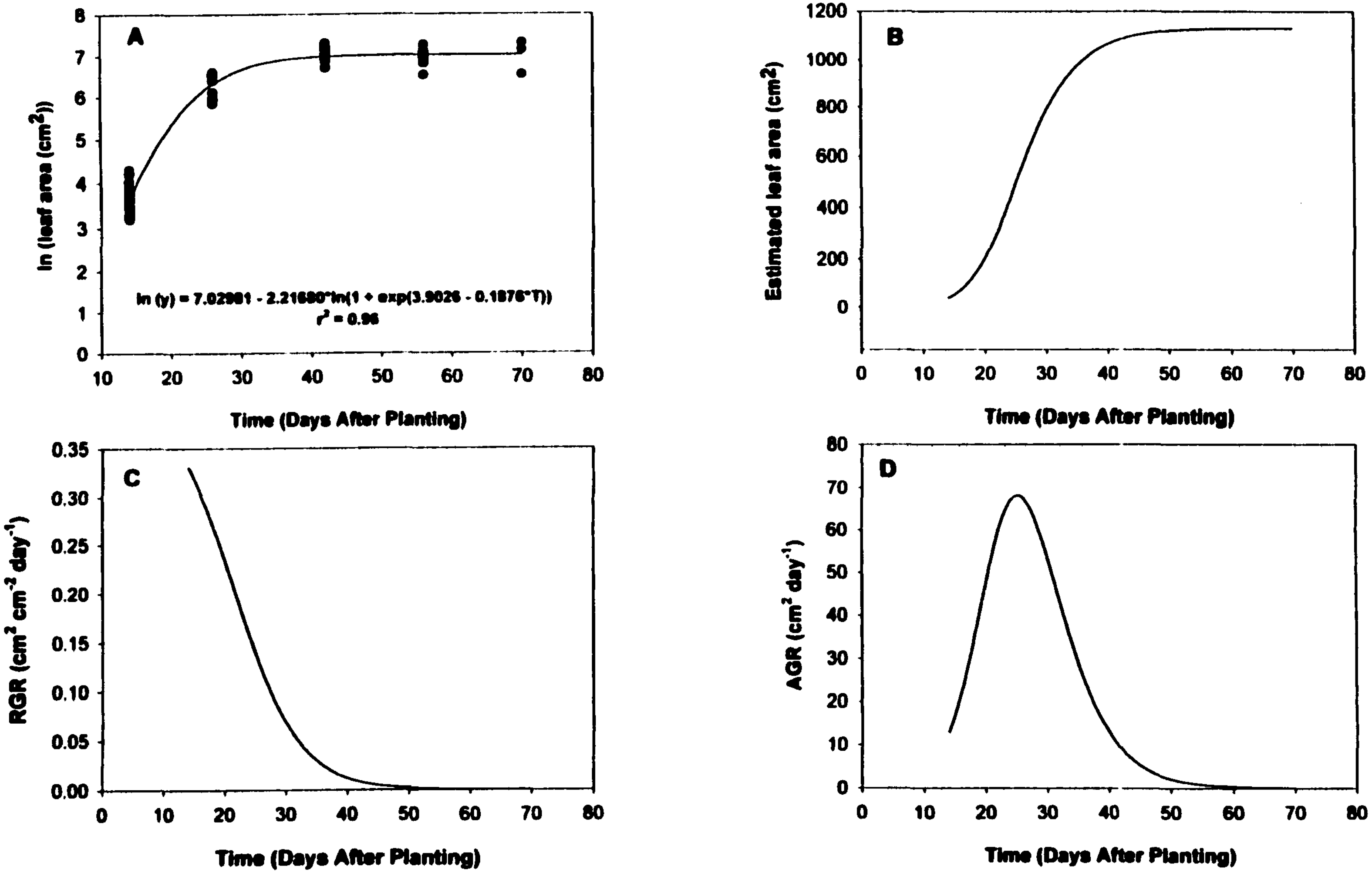

Figure 2.15. Richards function fitted curve (A), estimated leaf area (B), leaf area relative growth rate (C), and leaf area absolute growth rate (D) of Oregon-Grand Island olive seeded proso millet biotype.

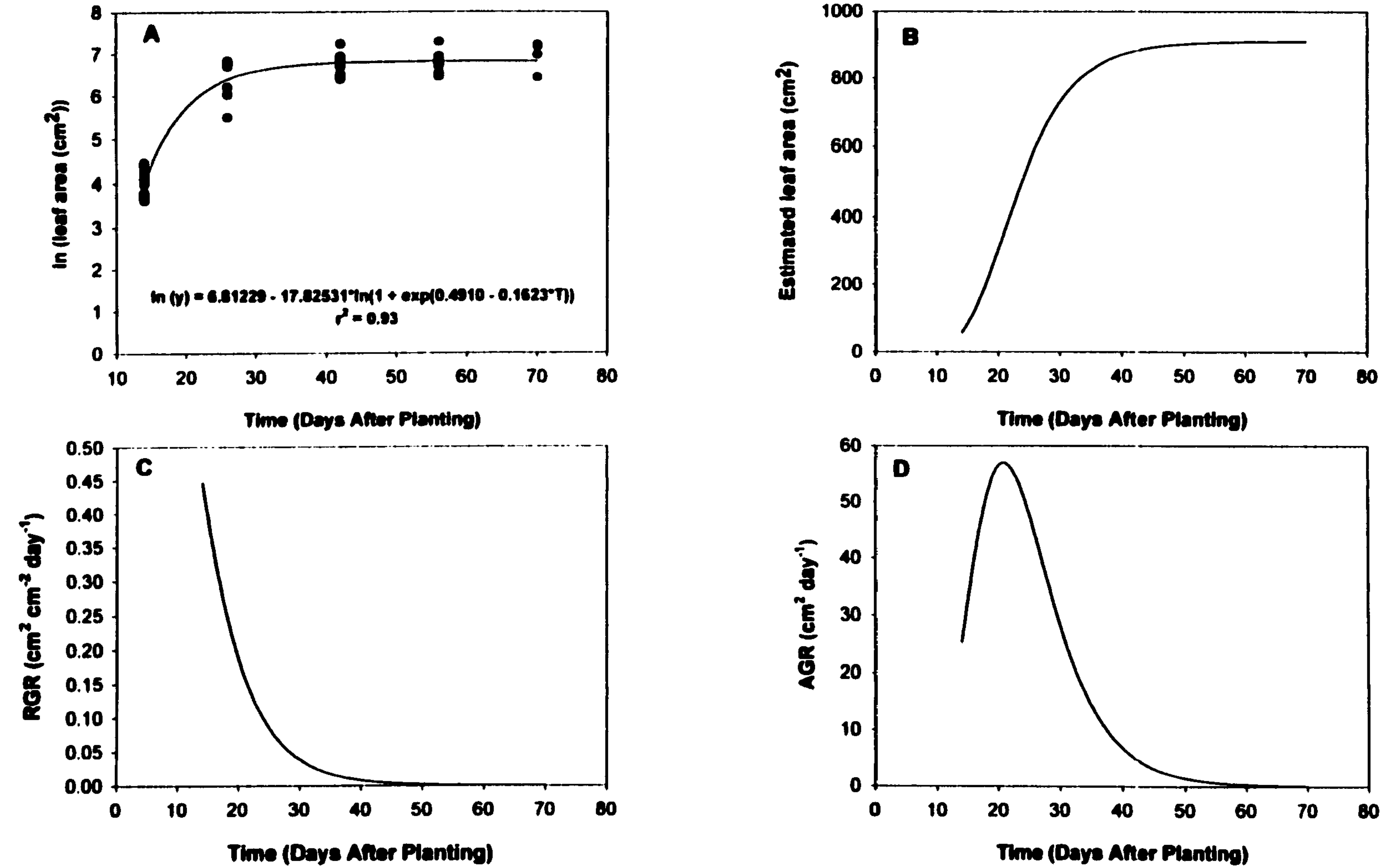

Figure 2.16. Richards function fitted curve (A), estimated leaf area (B), leaf area relative growth rate (C), and leaf area absolute growth rate (D) of Ontario, Canada-Huron County black seeded proso millet biotype.

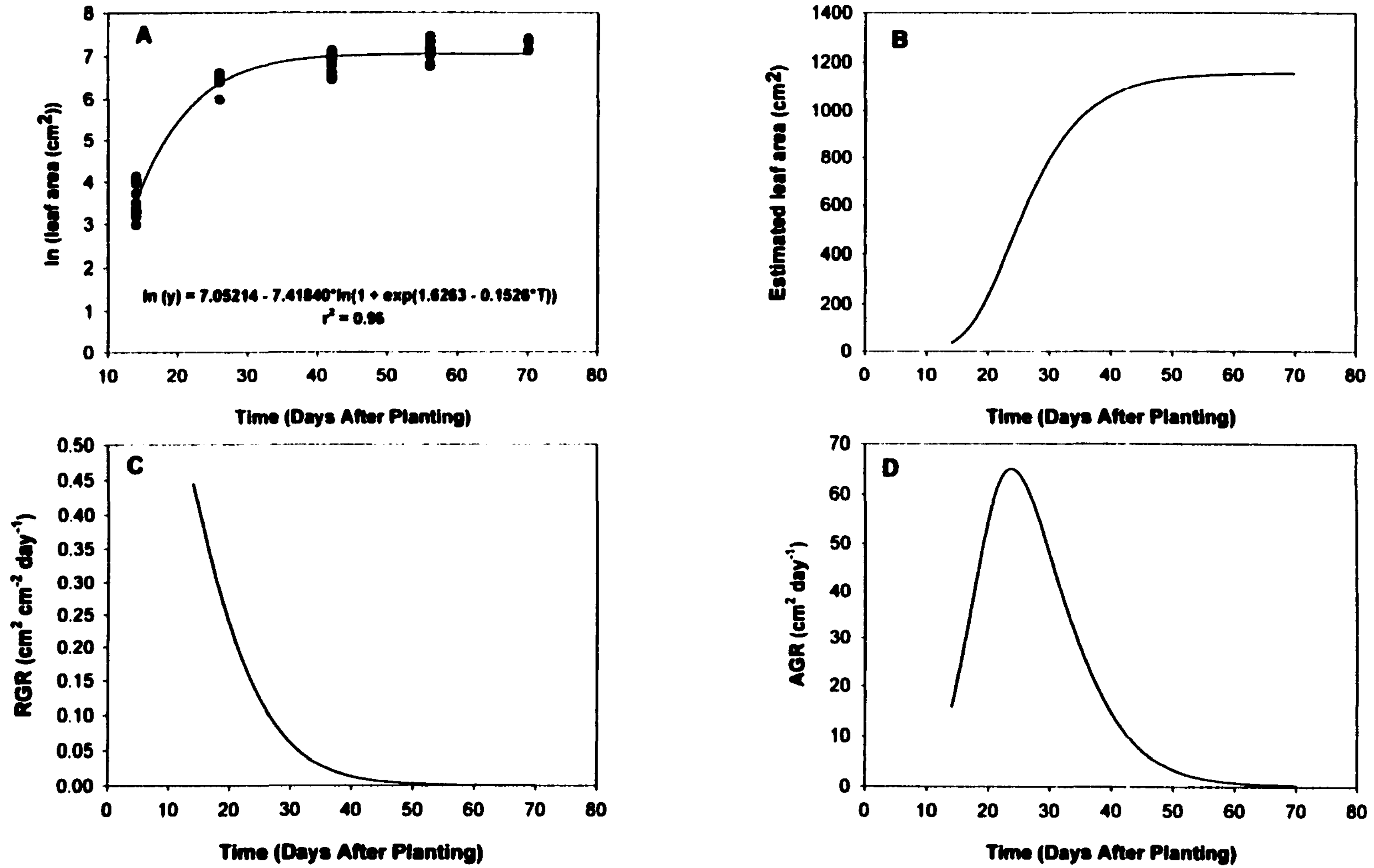

Figure 2.17. Richards function fitted curve (A), estimated leaf area (B), leaf area relative growth rate (C), and leaf area absolute growth rate (D) of Nebraska-Panhandle tan seeded proso millet biotype.

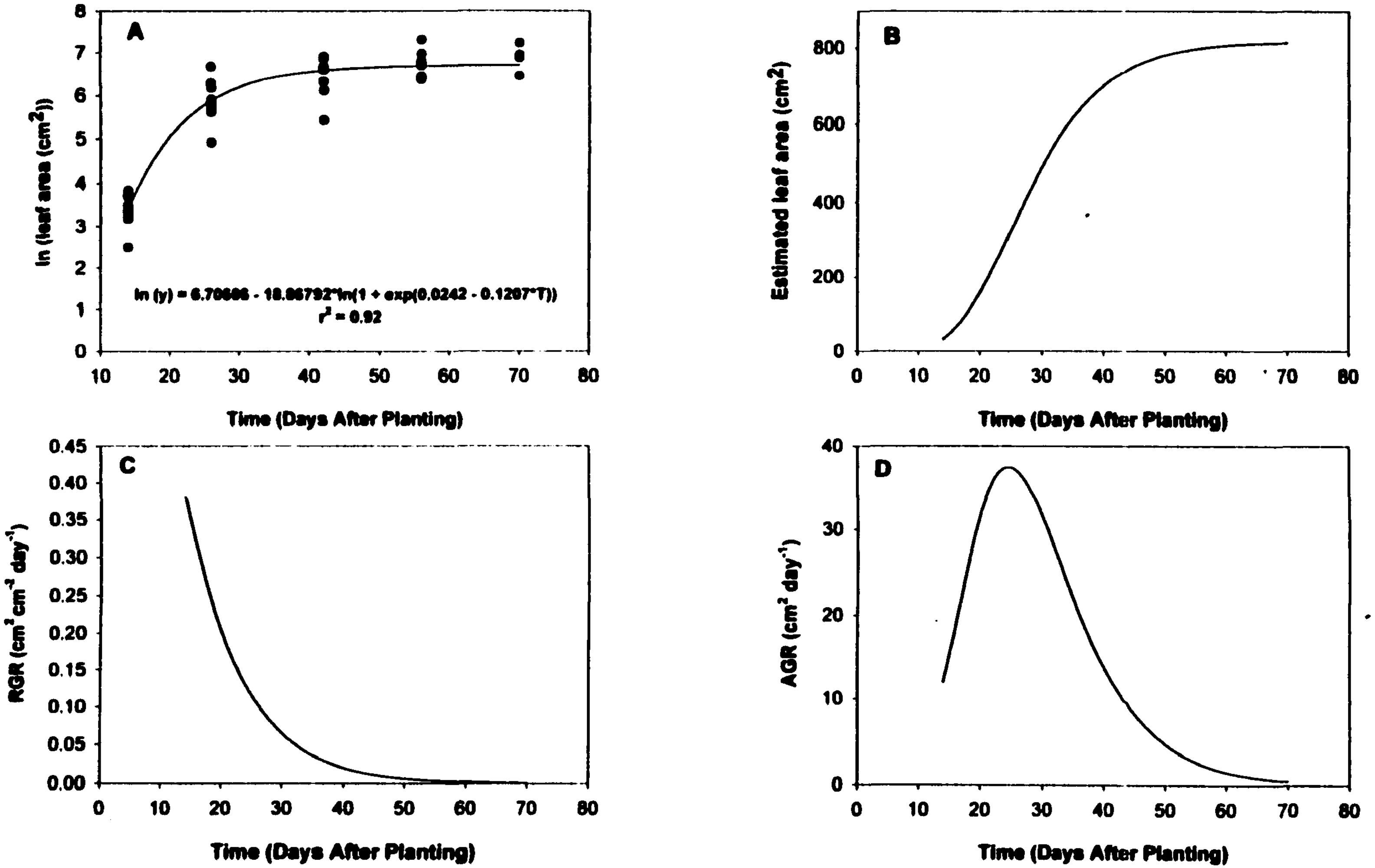

Figure 2.18. Richards function fitted curve (A), estimated leaf area (B), leaf area relative growth rate (C), and leaf area absolute growth rate (D) of Wyoming-Platte County brown seeded proso millet biotype.

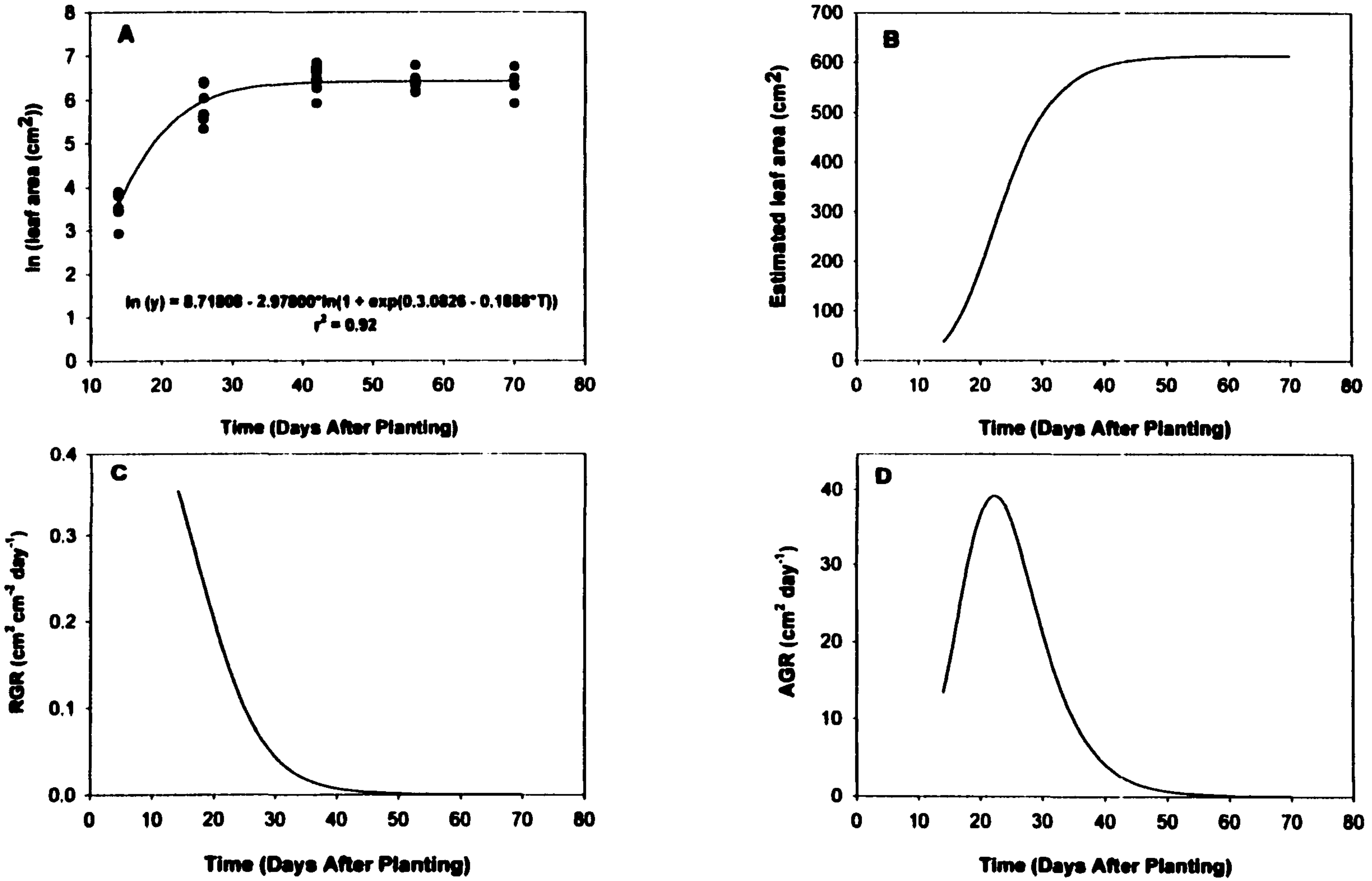

Figure 2.19. Richards function fitted curve (A), estimated leaf area (B), leaf area relative growth rate (C), and leaf area absolute growth rate (D) of Colorado-Weld County black seeded proso millet biotype.

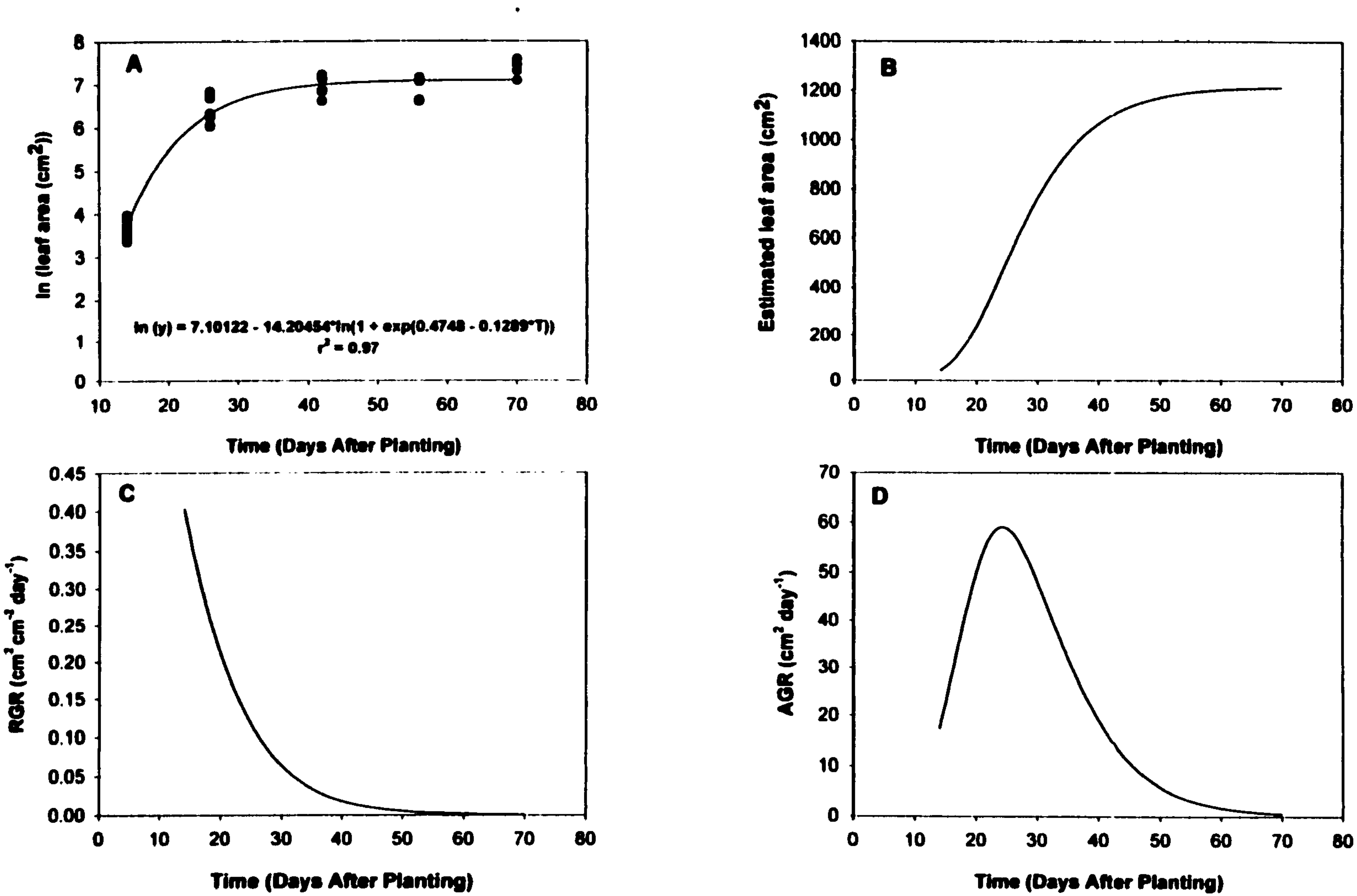

Figure 2.20. Richards function fitted curve (A), estimated leaf area (B), leaf area relative growth rate (C), and leaf area absolute growth rate (D) of Colorado-Weld County tan seeded proso millet biotype.

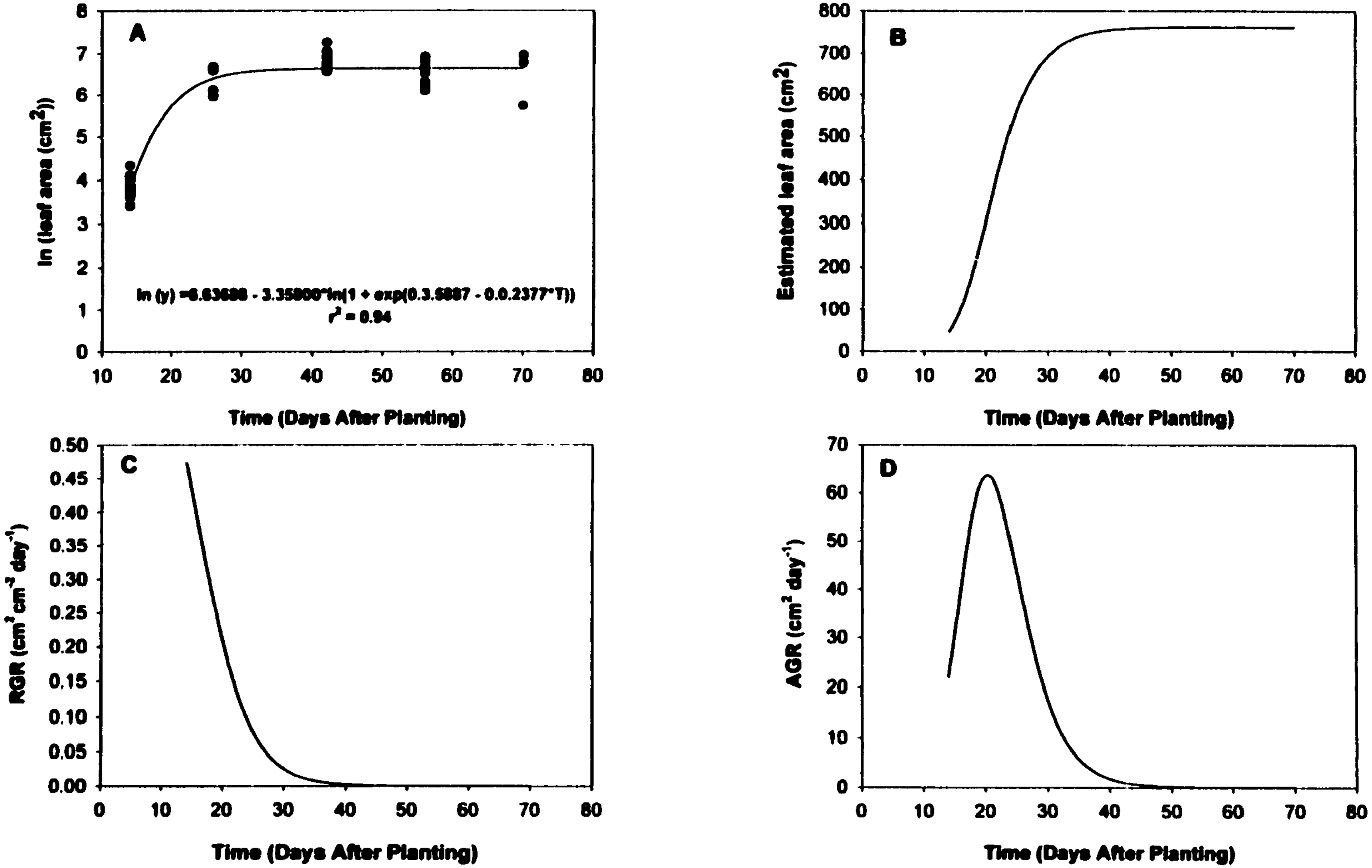

Figure 2.21. Richards function fitted curve (A), estimated leaf area (B), leaf area relative growth rate (C), and leaf area absolute growth rate (D) of South Dakota brown seeded proso millet biotype.

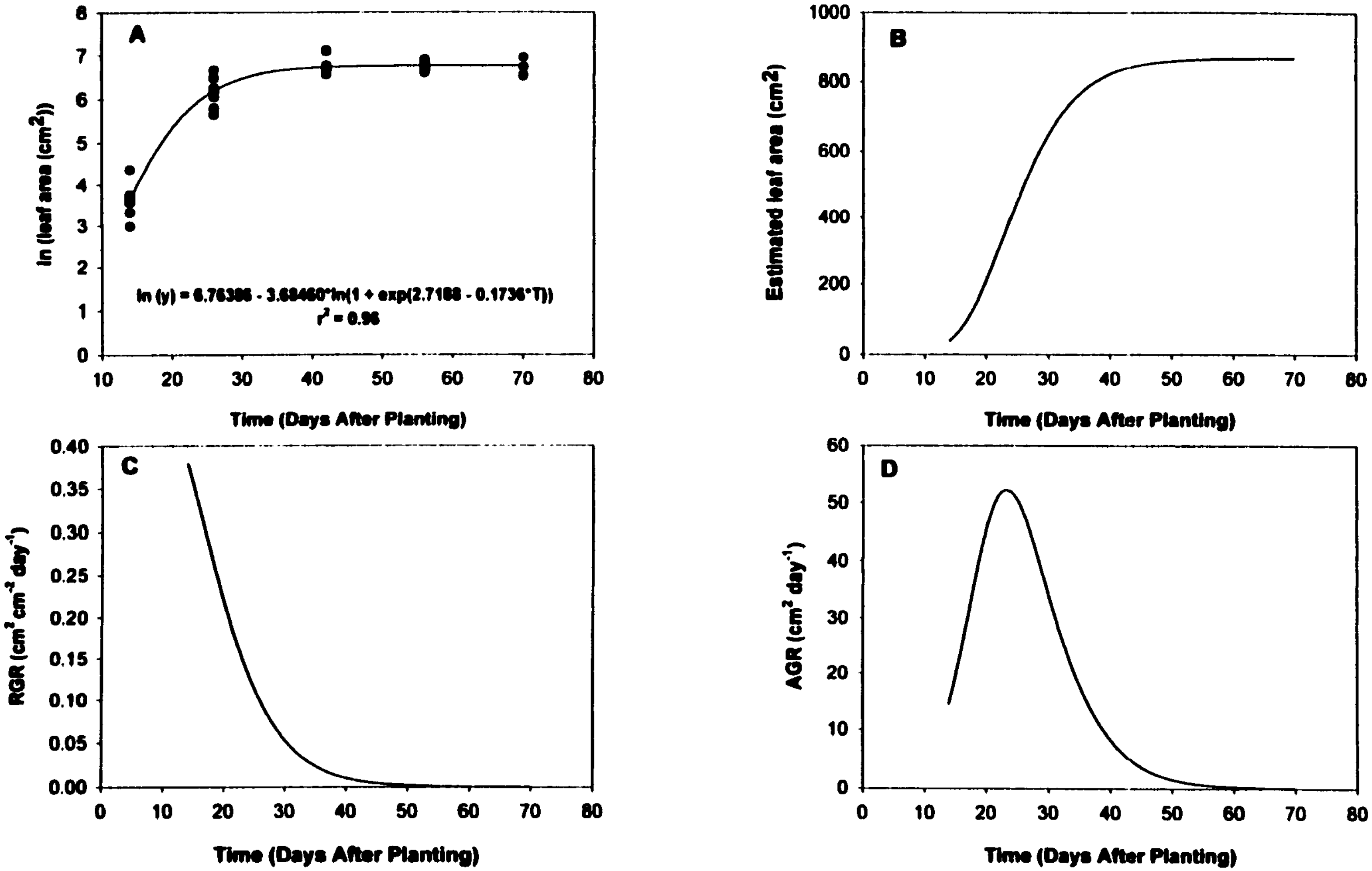

Figure 2.22. Richards function fitted curve (A), estimated leaf area (B), leaf area relative growth rate (C), and leaf area absolute growth rate (D) of Colorado white seeded proso millet biotype.

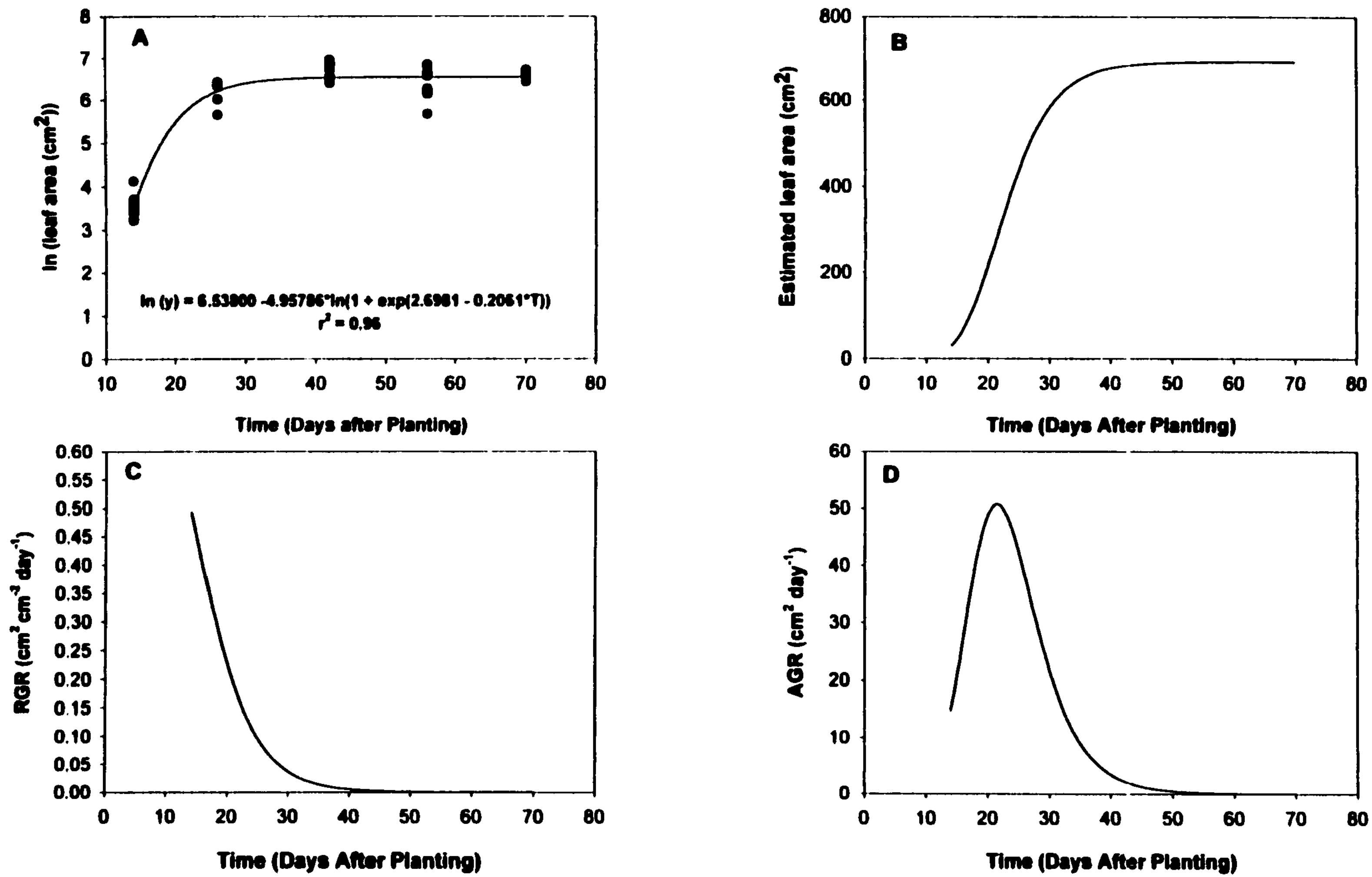

Figure 2.23. Richards function fitted curve (A), estimated leaf area (B), leaf area relative growth rate (C), and leaf area absolute growth rate (D) of Colorado orange seeded proso millet biotype.

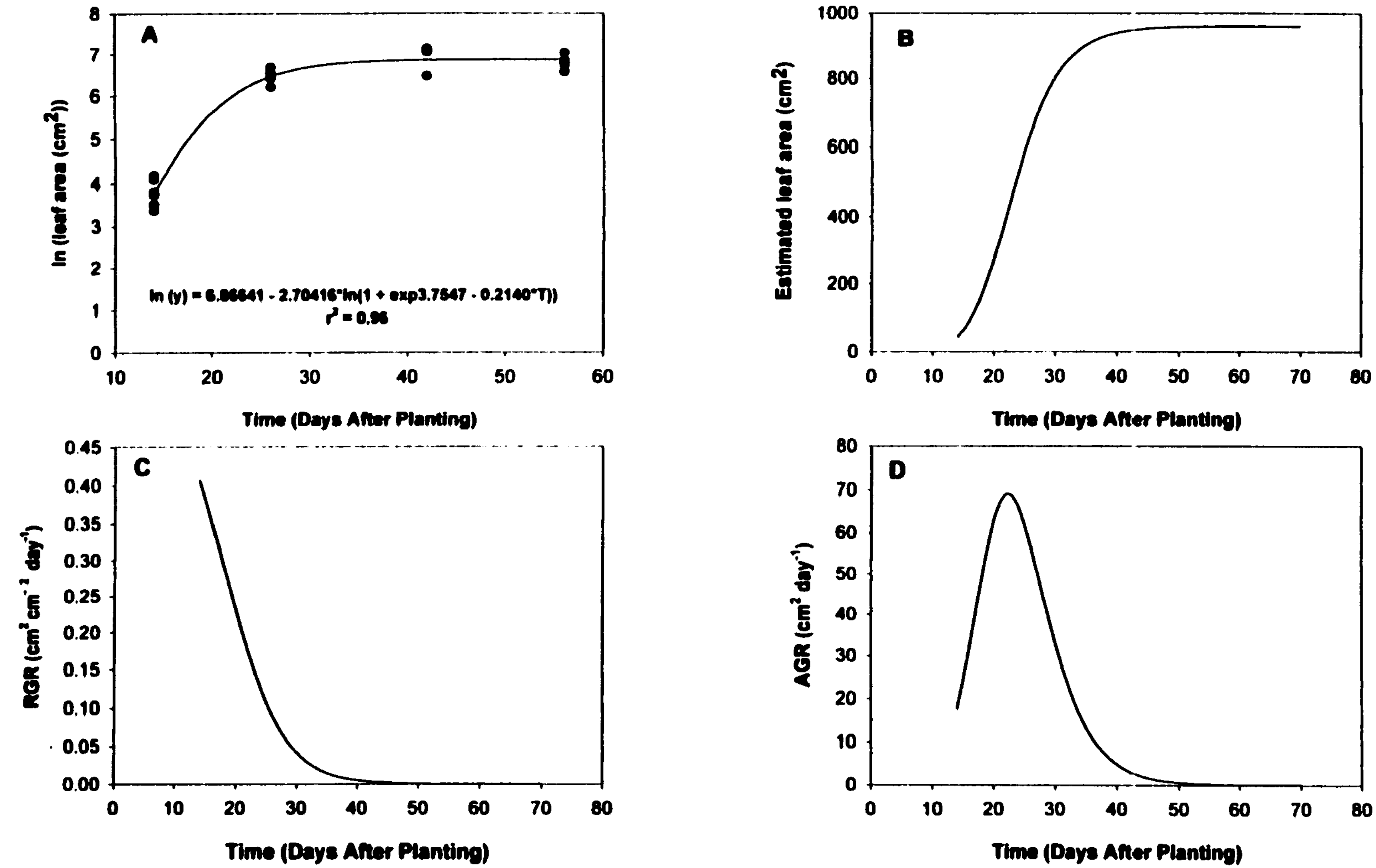

Figure 2.24. Richards function fitted curve (A), estimated leaf area (B), leaf area relative growth rate (C), and leaf area absolute growth rate (D) of Colorado-Larimer white seeded proso millet biotype.

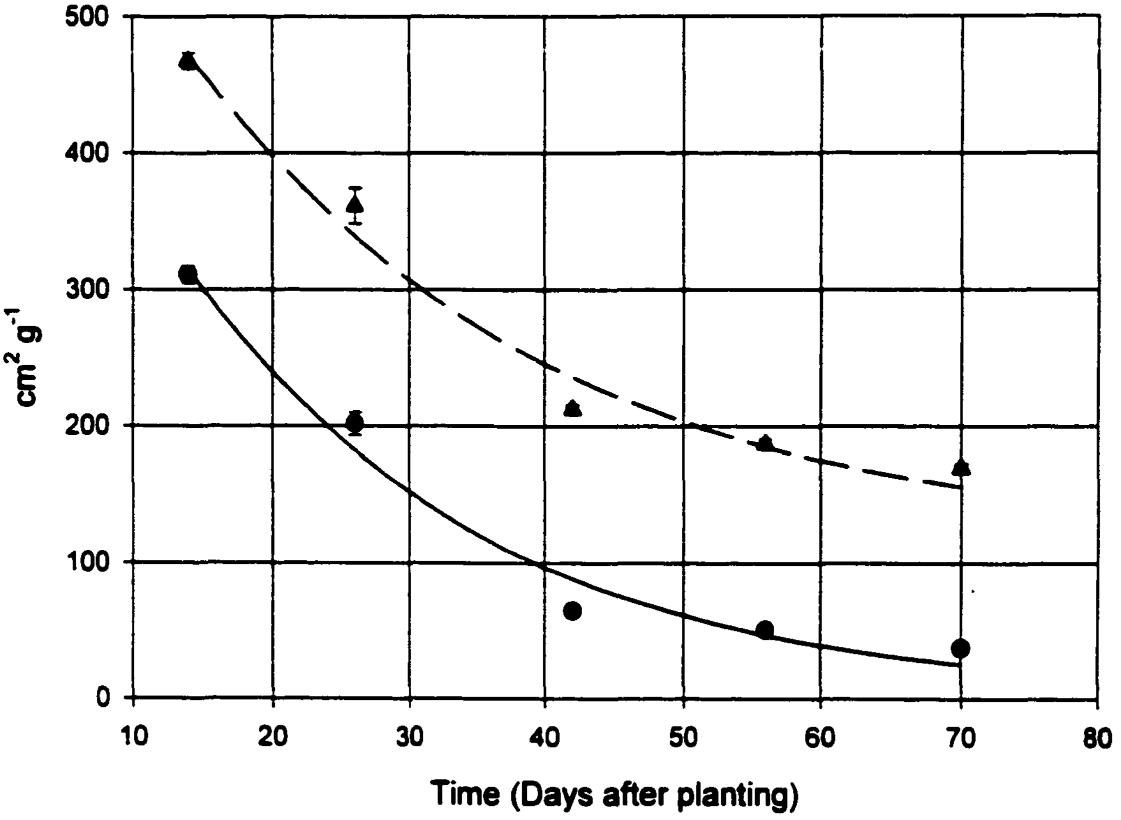

Figure 2.25. Leaf area ratio (- - y = 0.6909 + 594.8806*exp (-0.0456*DAP), r^2 = 0.81) and specific leaf area (— y = 114.9031 + 615.1196*exp (-0.0388*DAP), r^2 = 0.75) of proso millet biotypes.

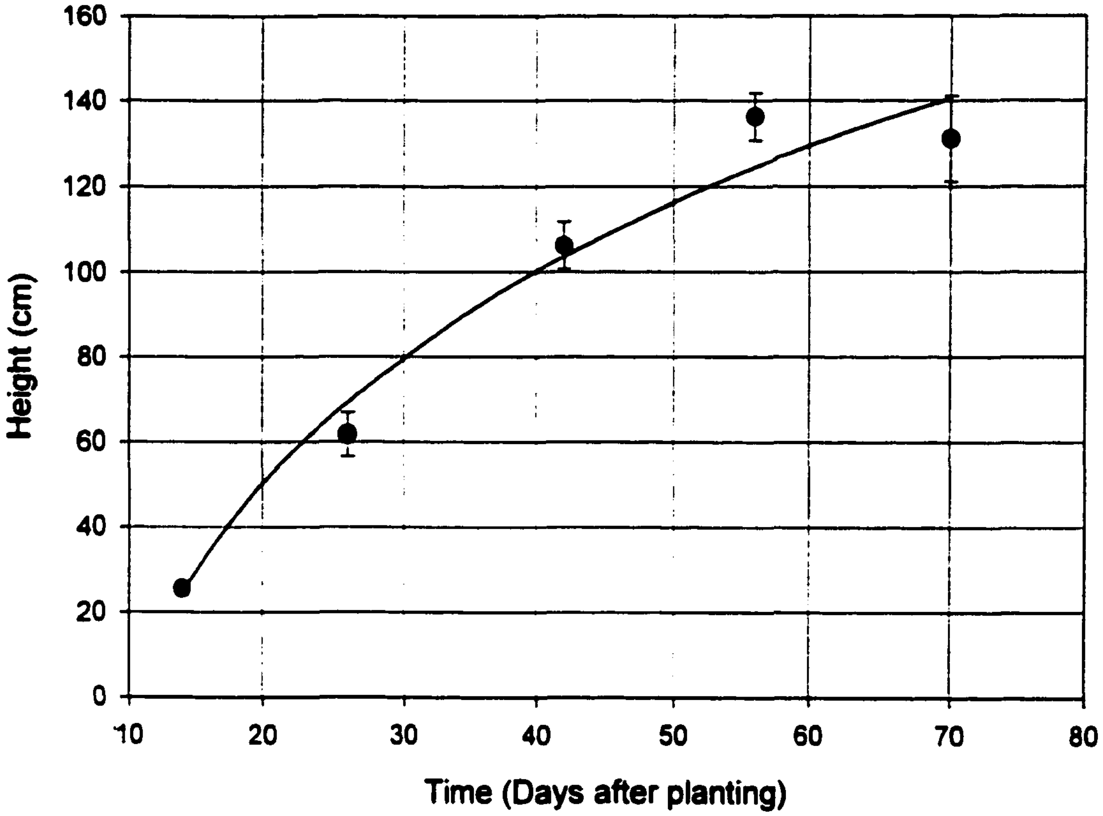

Figure 2.26. Minnesota-Cambridge olive seeded height over time ($y = -165.6990 + 72.0883*\ln(DAP)$, $r^2 = 0.87$).

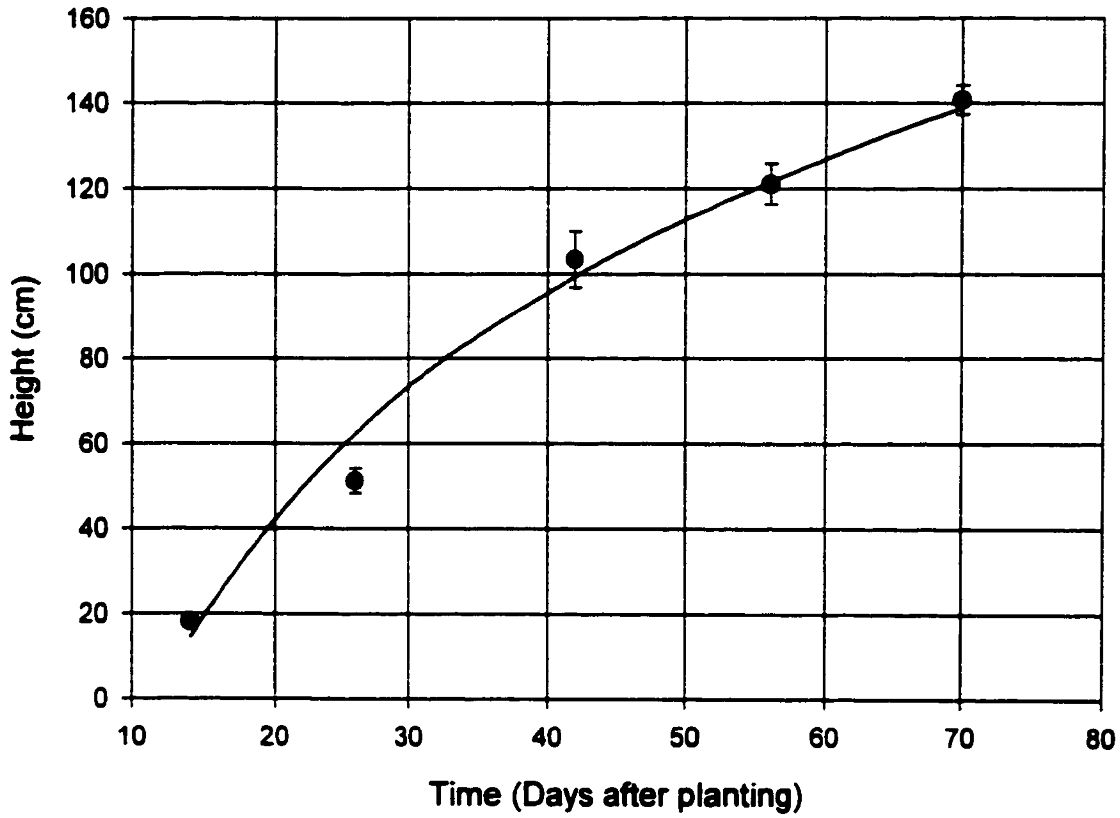

Figure 2.27. Canada-Rosemount black seeded height over time ($y = -189.4327 + 77.2631*\ln(DAP)$, $r^2 = 0.94$).

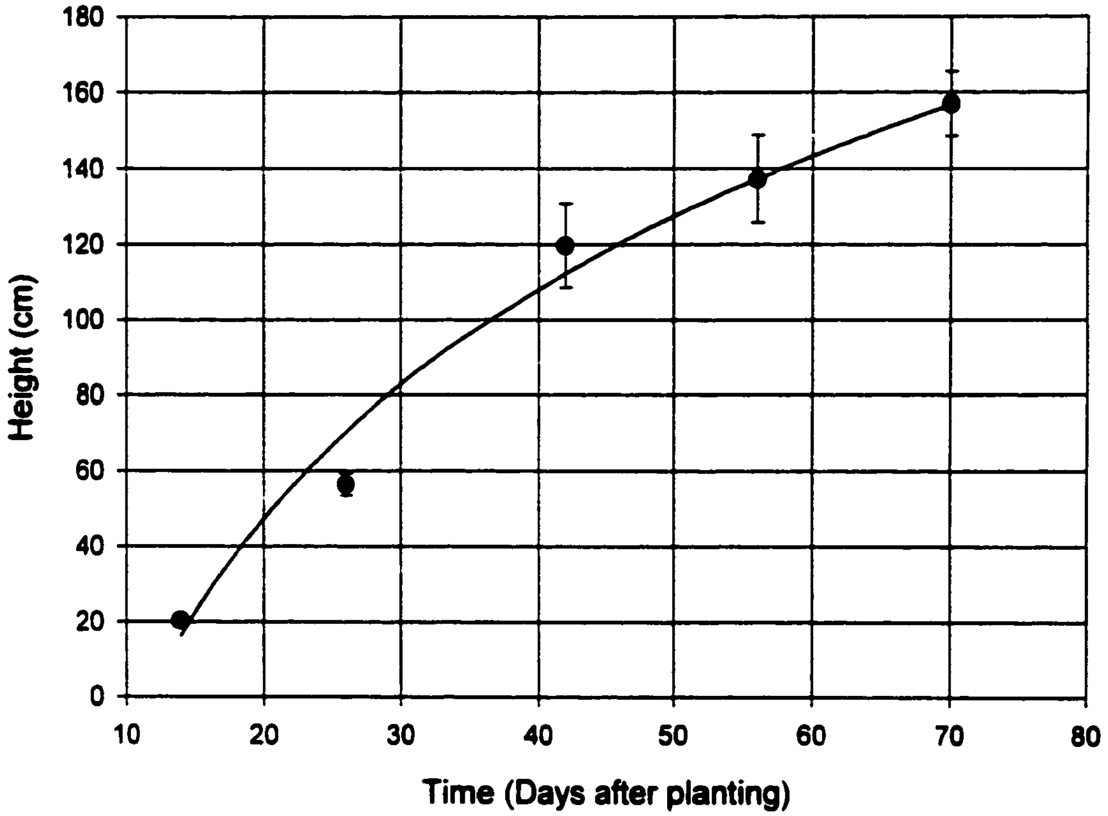

Figure 2.28. Oregon-Grand Island olive seeded height over time ($y = -214.5222 + 87.4190*\ln(DAP)$, $r^2 = 0.85$).

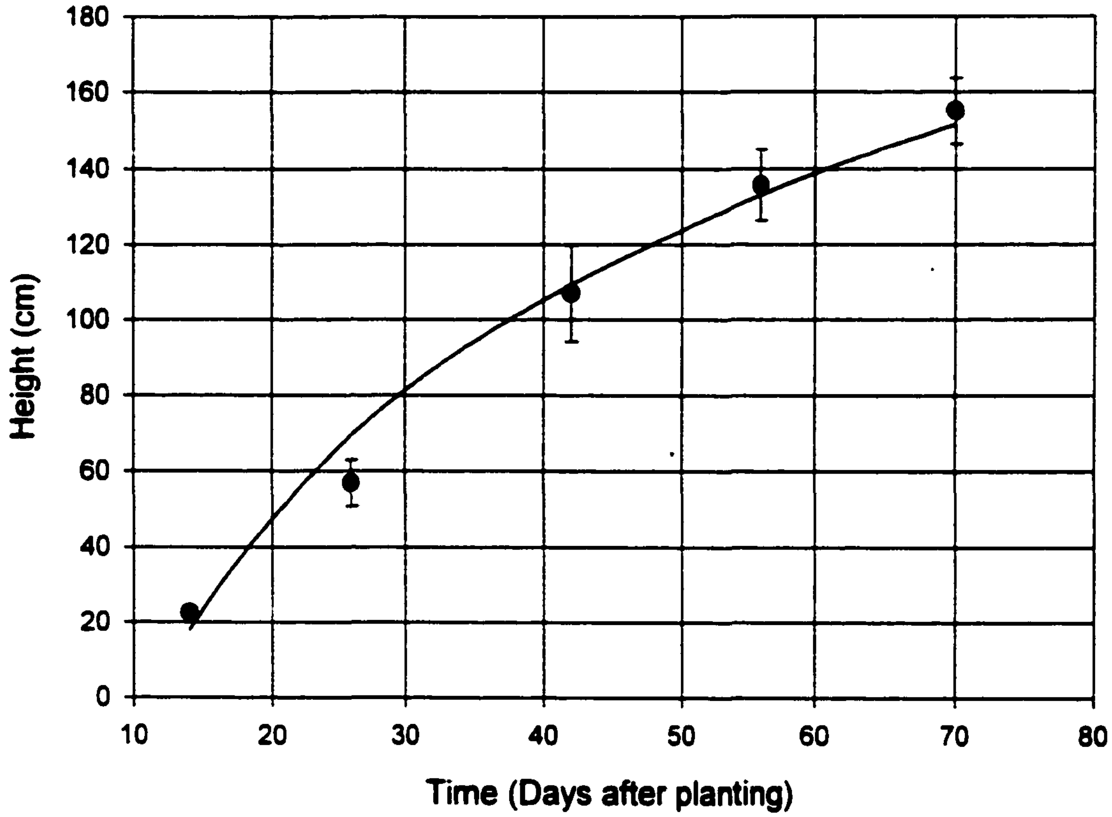

Figure 2.29. Ontario, Canada-Huron County black seeded height over time ($y = -201.6004 + 83.1568*\ln(DAP)$, $r^2 = 0.84$).

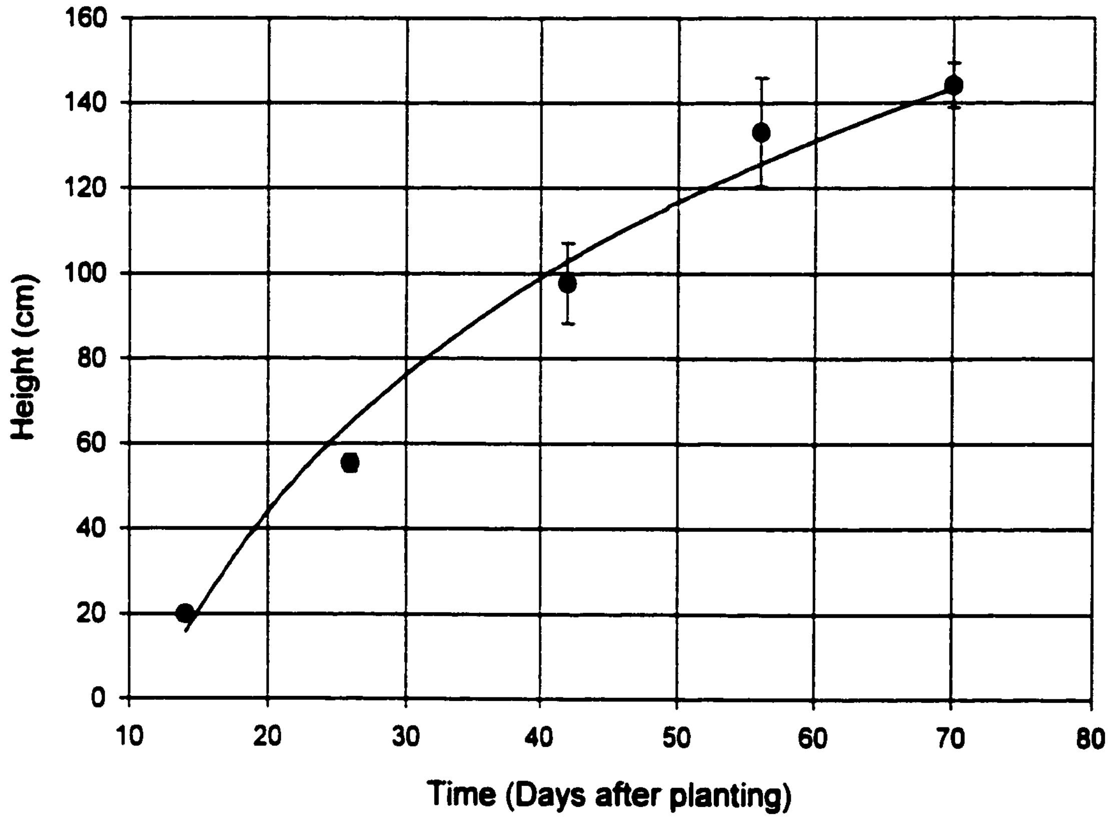

Figure 2.30. Nebraska-Panhandle County tan seeded height over time ($y = -193.7277 + 79.3445*\ln(DAP)$, $r^2 = 0.85$).

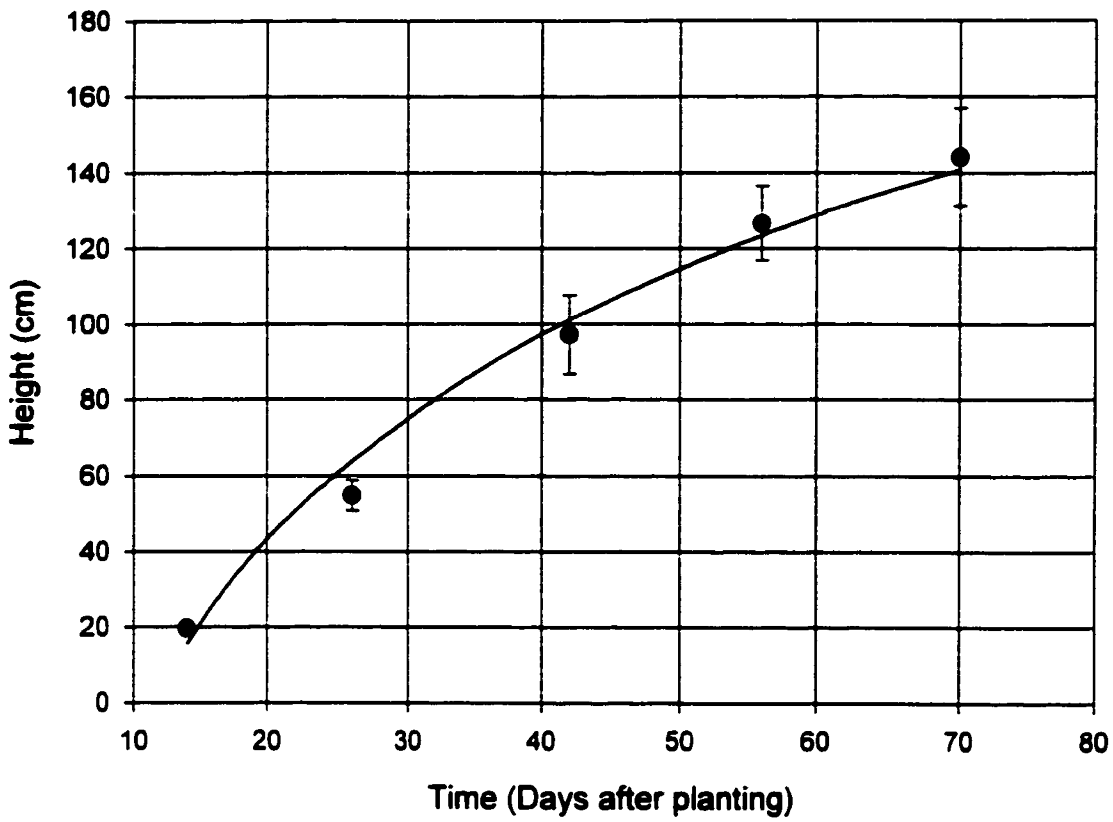

Figure 2.31. Wyoming-Platte County brown seeded height over time ($y = -190.1086 + 77.8998 \cdot \ln(DAP)$, $r^2 = 0.81$).

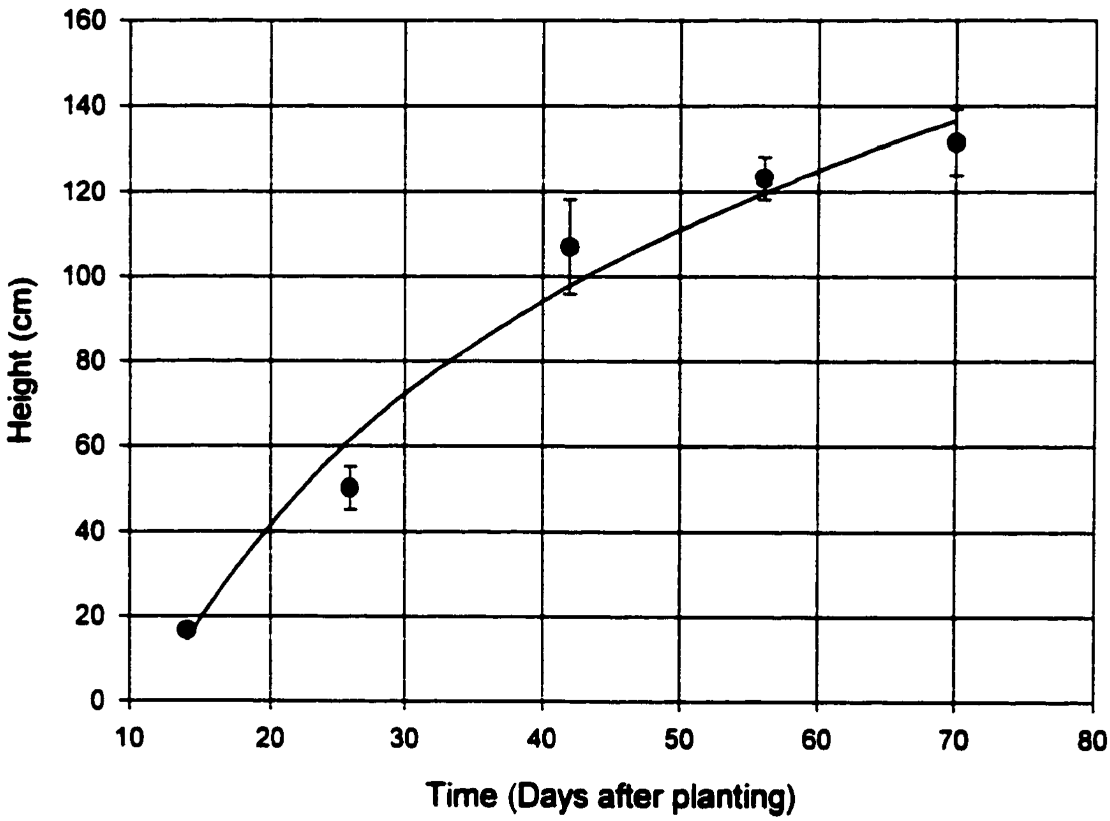

Figure 2.32. Colorado-Weld County black seeded height over time ($y = -186.0373 + 75.9272*\ln(DAP)$, $r^2 = 0.86$).

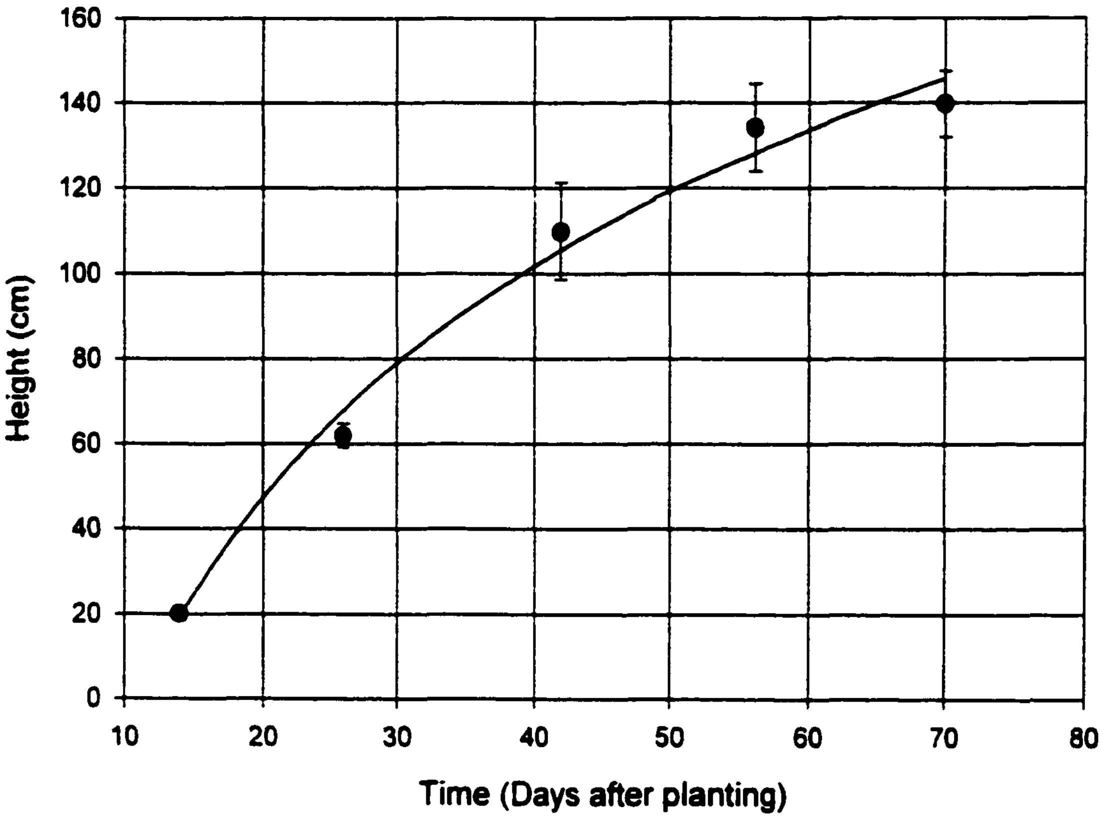

Figure 2.33. Colorado-Weld County tan seeded height over time ($y = -188.2569 + 78.611*\ln(DAP)$, $r^2 = 0.84$).

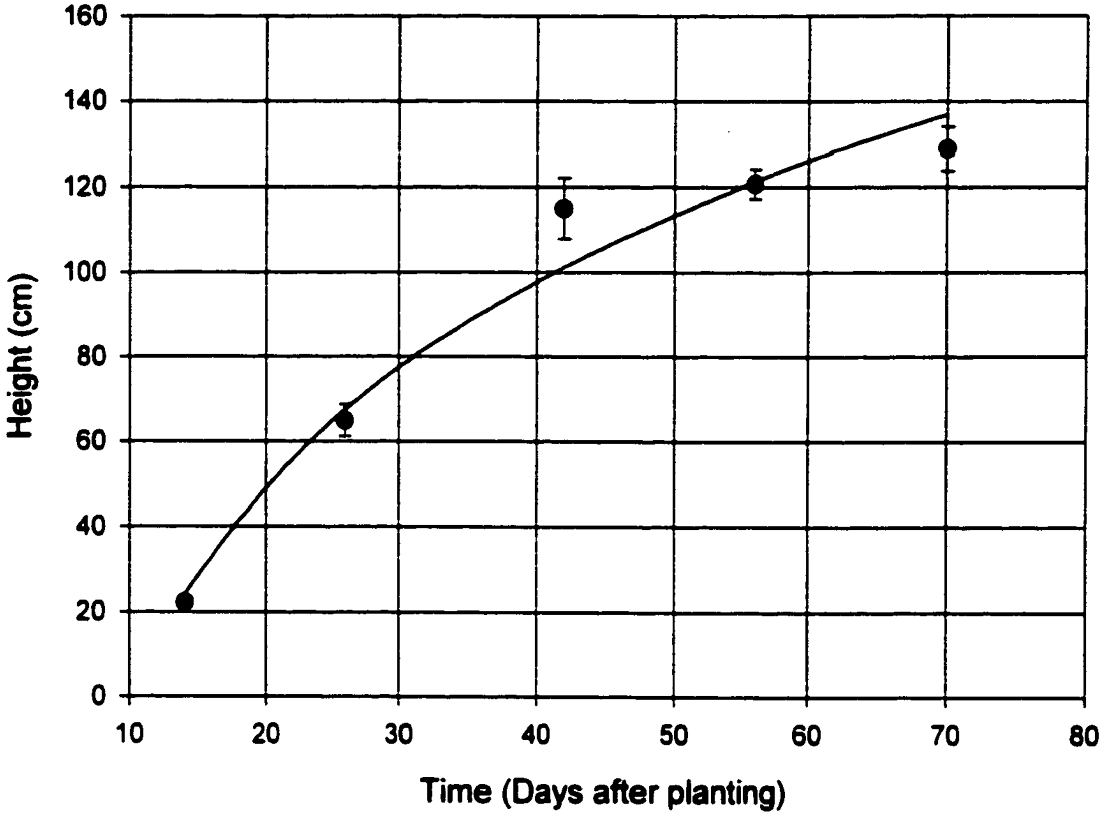

Figure 2.34. South Dakota brown seeded height over time ($y = -161.1654 + 70.1657*\ln(DAP)$, $r^2 = 0.91$).

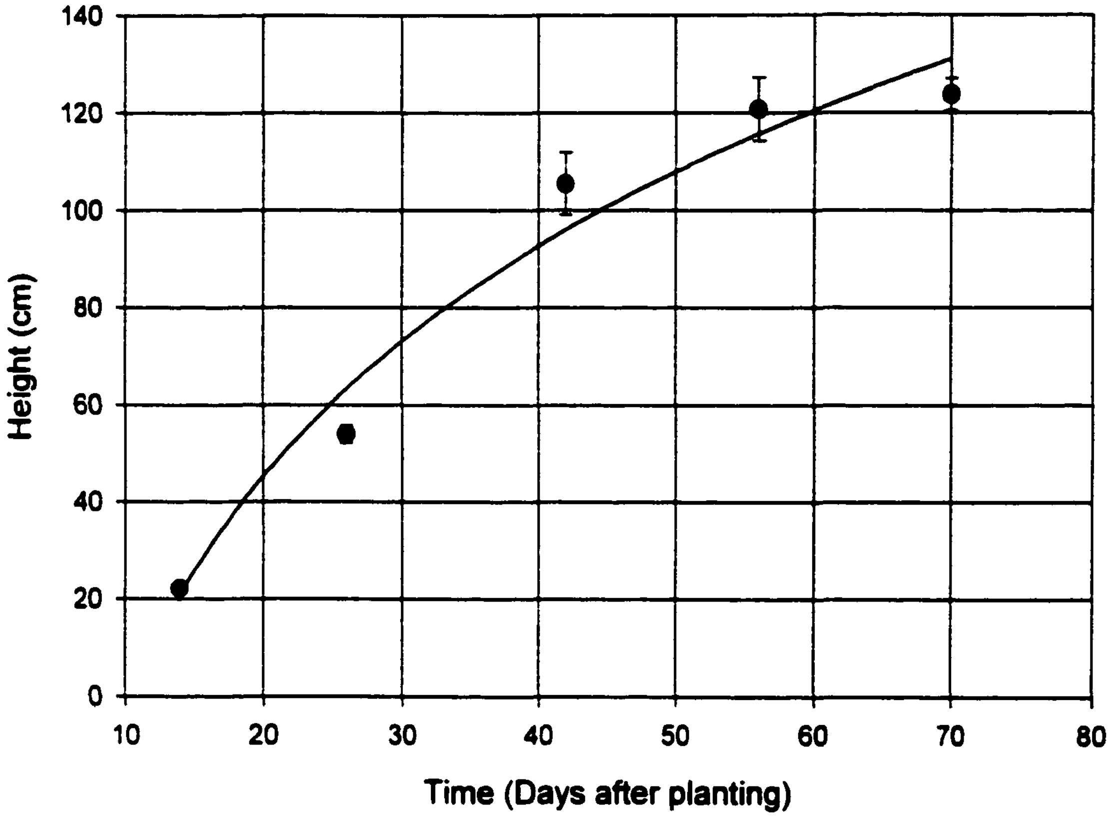

Figure 2.35. Colorado brown seeded height over time ($y = -159.5893 + 63.3900*\ln(DAP)$, $r^2 = 0.91$).

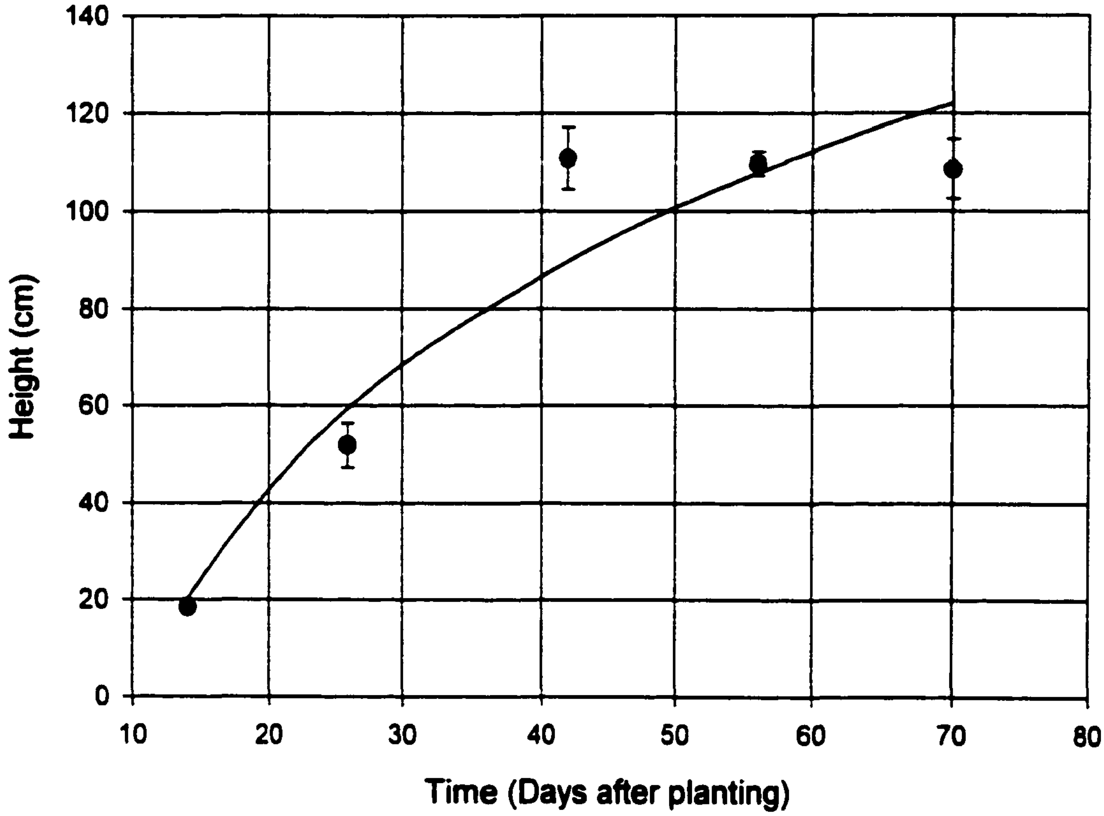

Figure 2.36. Colorado orange seeded height over time ($y = -146.9473 + 63.3157*\ln(DAP)$, $r^2 = 0.85$).

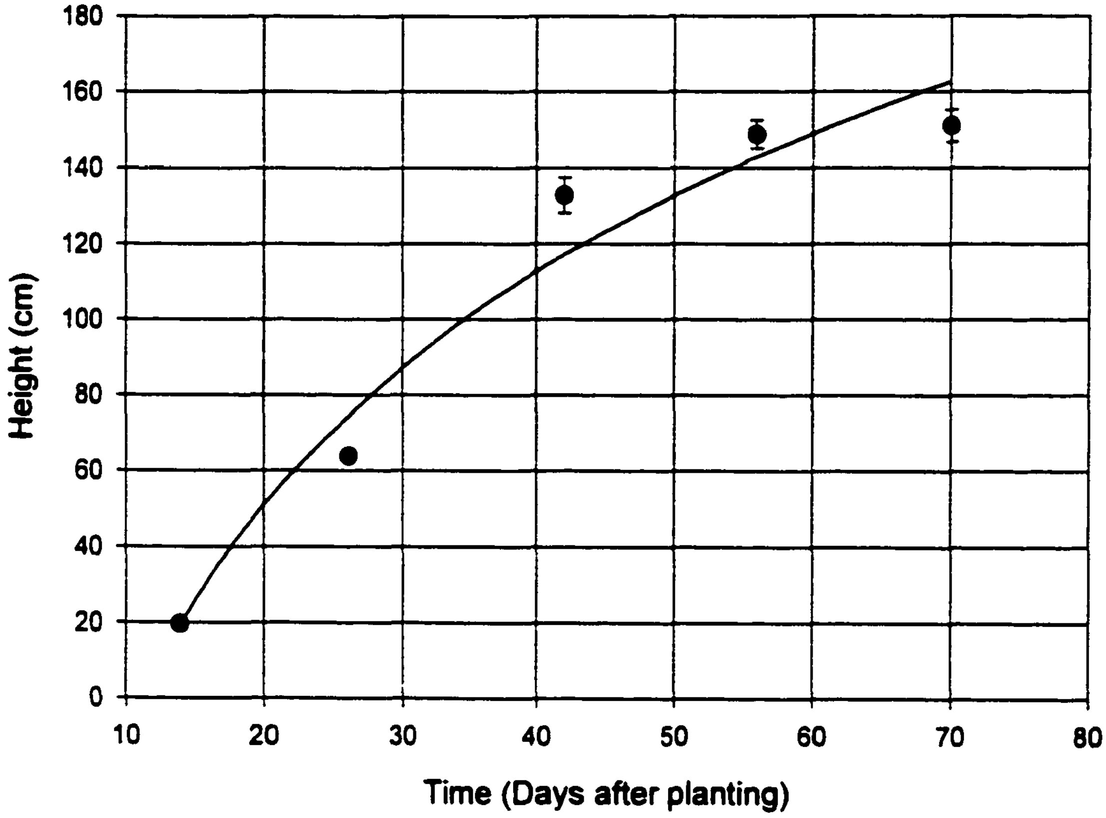

Figure 2.37. Colorado-Larimer white seeded height over time ($y = -216.2642 + 89.2012*\ln(DAP)$, $r^2 = 0.95$).

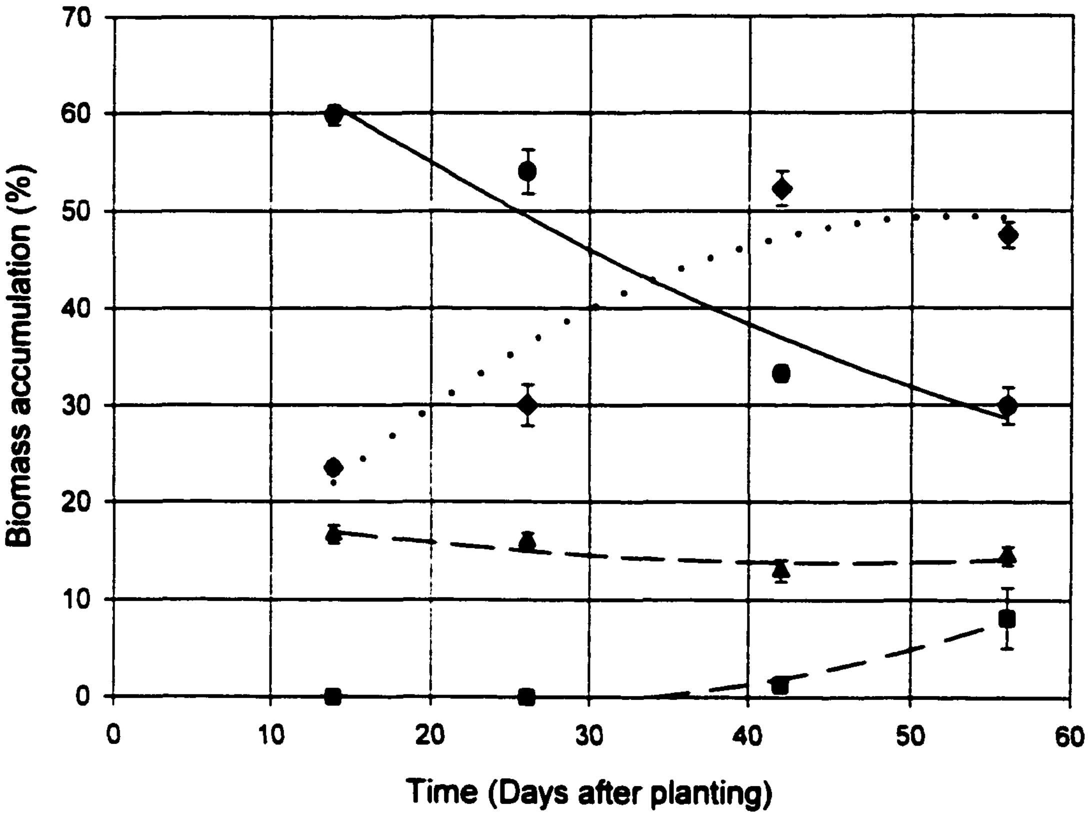

Figure 2.38. Percent of total biomass accumulation of Minnesota-Cambridge olive seeded proso millet biotype. Leaf (— $y = 76.4843 - 1.1996*DAP + 0.0061*DAP^2$, $r^2 = 0.87$), stem (···· $y = -1.3679 + 1.9201*DAP - 0.0181*DAP^2$, $r^2 = 0.81$), root (- - $y = 20.4125 - 0.2911*DAP + 0.032*DAP^2$, $r^2 = 0.13$), and reproductive tissue (- · - $y = 4.4711 - 0.4294*DAP + 0.0088*DAP^2$, $r^2 = 0.36$).

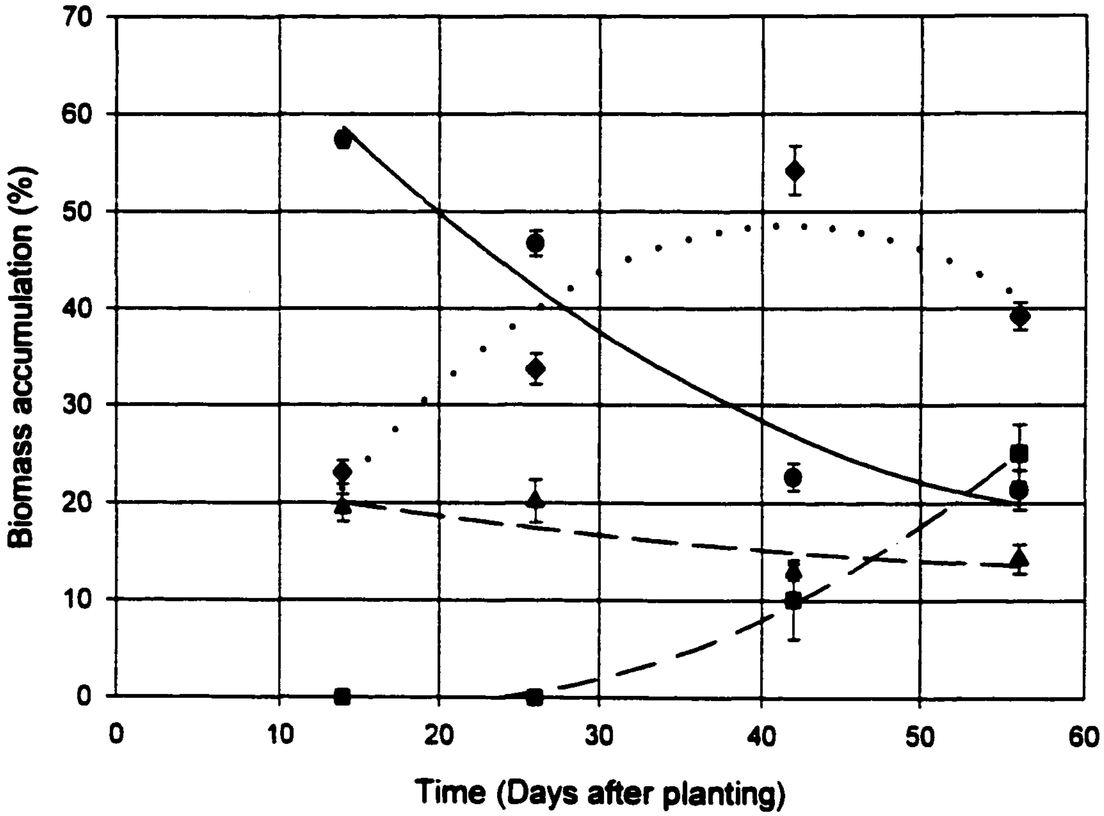

Figure 2.39. Percent of total biomass accumulation of Canada-Rosemount black seeded proso millet biotype. Leaf (— $y = 83.4831 - 1.9853*DAP + 0.0152*DAP^2$, $r^2 = 0.91$), stem (····· $y = -13.0494 + 2.9593*DAP - 0.0355*DAP^2$, $r^2 = 0.73$), root (– – $y = 23.8939 - 0.3045*DAP + 0.0022*DAP^2$, $r^2 = 0.22$), and reproductive tissue (– · – $y = 5.7260 - 0.6752*DAP + 0.0183*DAP^2$, $r^2 = 0.71$).

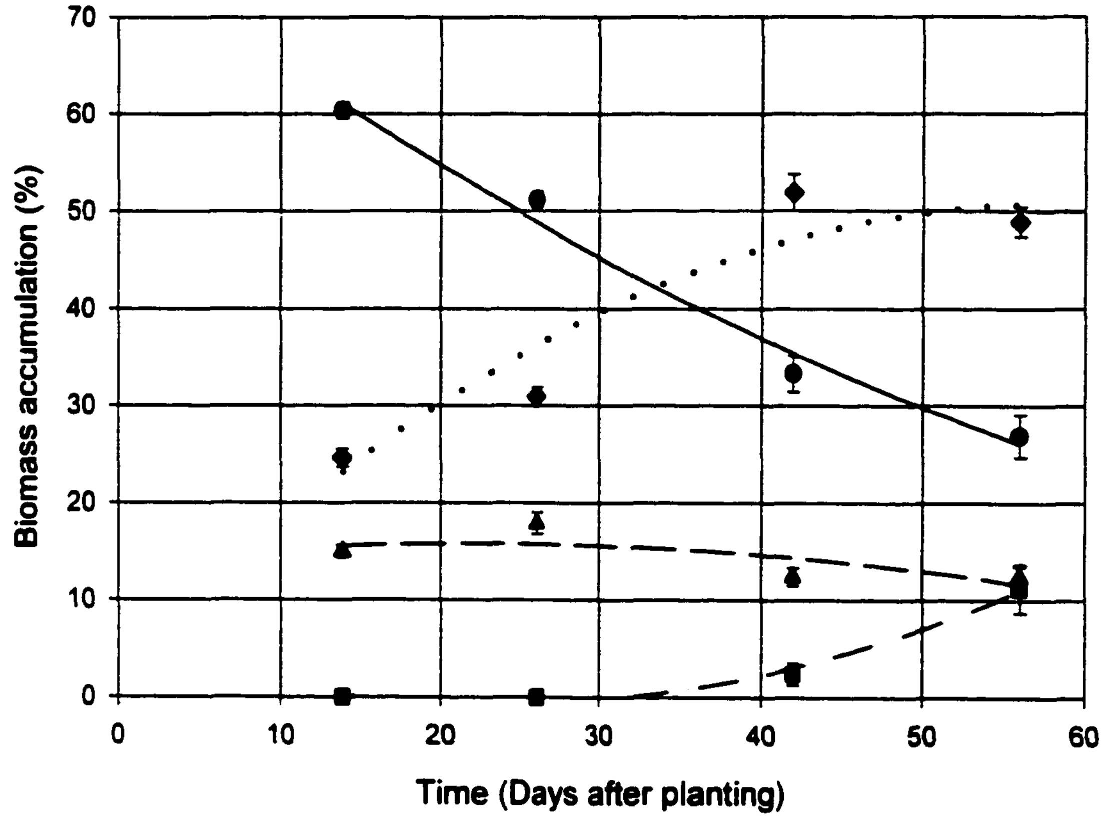

Figure 2.40. Percent of total biomass accumulation of Oregon-Grand Island olive seeded proso millet biotype. Leaf (— $y = 77.4512 - 1.2565*DAP + 0.0061*DAP^2$, $r^2 = 0.90$), stem (····· $y = 2.7515 + 1.6600*DAP - 0.0144*DAP^2$, $r^2 = 0.82$), root (- - $y = 14.0165 + 0.1608*DAP - 0.036*DAP^2$, $r^2 = 0.17$), and reproductive tissue (- · - $y = 5.2583 - 0.5165*DAP + 0.0110*DAP^2$, $r^2 = 0.60$).

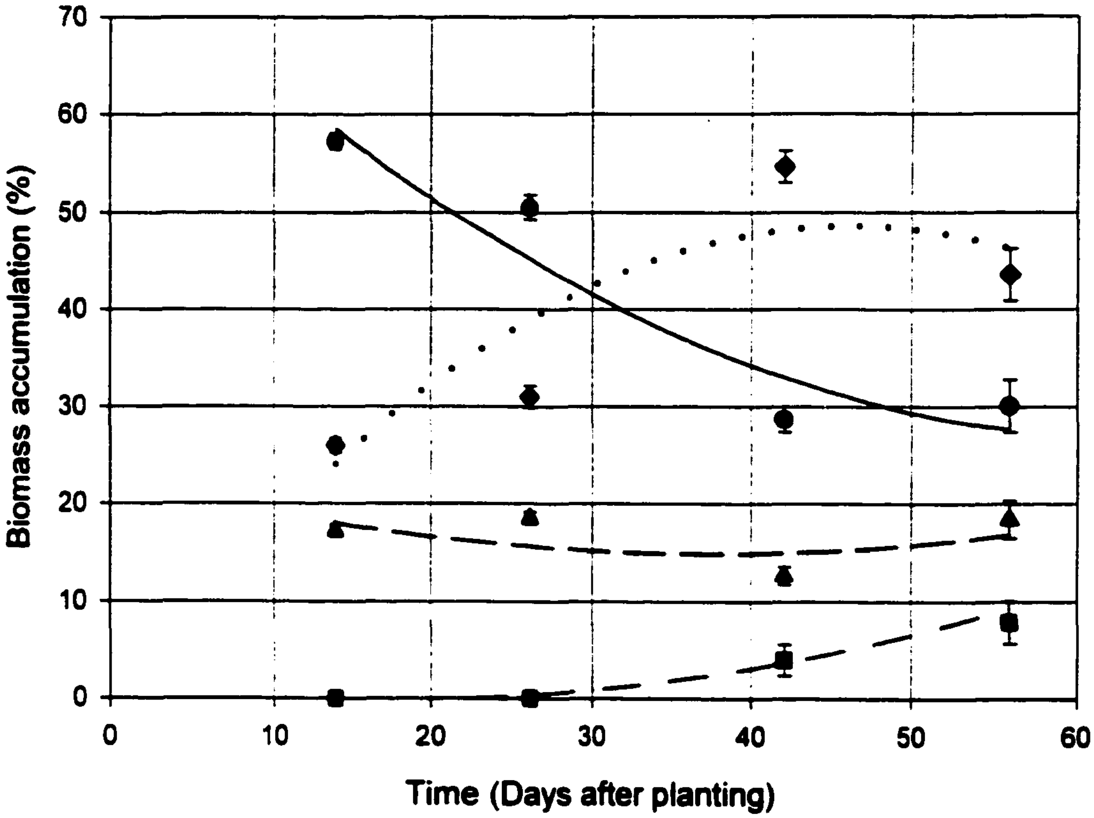

Figure 2.41. Percent of total biomass accumulation of Ontario, Canada-Huron County black seeded proso millet biotype. Leaf (— $y = 78.6213 - 1.6116*DAP + 0.0125*DAP^2$, $r^2 = 0.83$), stem (····· $y = -1.9724 + 2.1921*DAP - 0.0238*DAP^2$, $r^2 = 0.69$), root (- - $y = 22.7580 - 0.4202*DAP + 0.0056*DAP^2$, $r^2 = 0.04^{ns}$), and reproductive tissue (- · - $y = 1.7255 - 0.2169*DAP + 0.0063*DAP^2$, $r^2 = 0.45$).

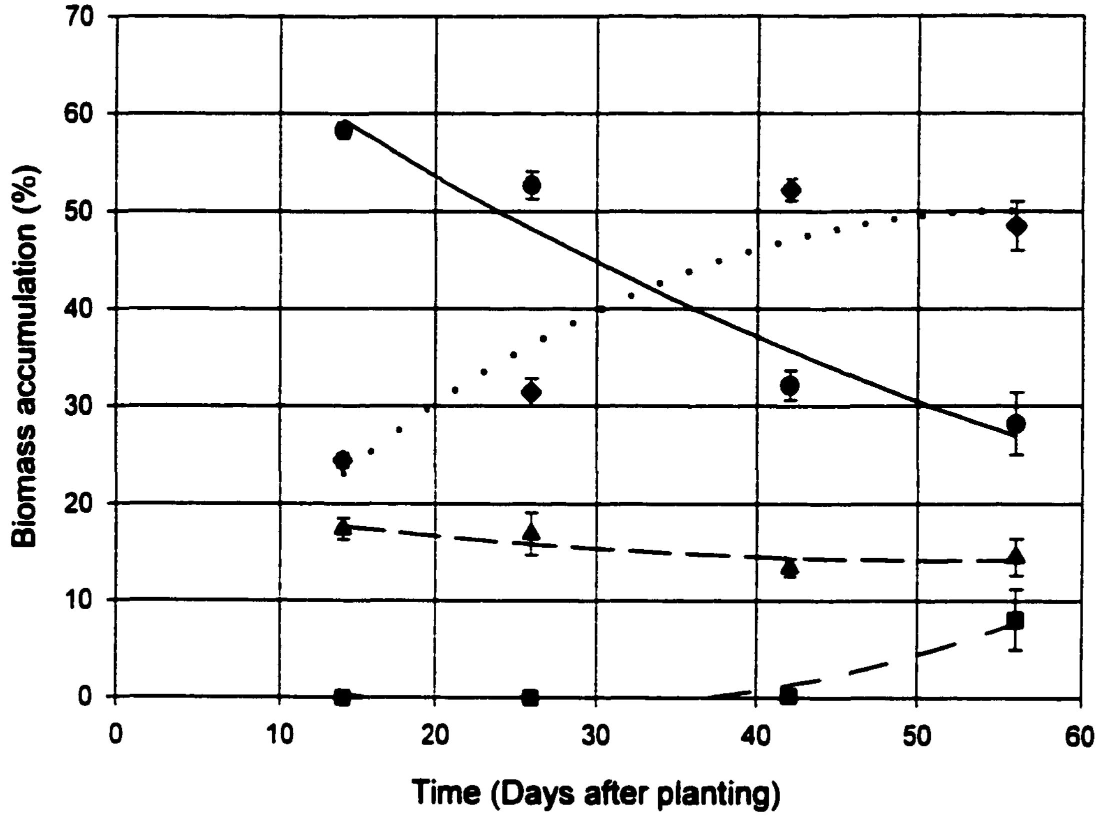

Figure 2.42. Percent of total biomass accumulation of Nebraska-Panhandle Center tan seeded proso millet biotype. Leaf (— y = 74.2483 - 1.1377*DAP + 0.0061*DAP^2, r^2 = 0.83), stem (····· y = 1.9707 + 1.7203*DAP - 0.0154*DAP^2, r^2 = 0.80), root (- - y = 20.6403 - 0.2458*DAP + 0.0023*DAP^2, r^2 = 0.05^{ns}), and reproductive tissue (- · - y = 5.7556 - 0.5290*DAP + 0.0101*DAP^2, r^2 = 0.0.37).

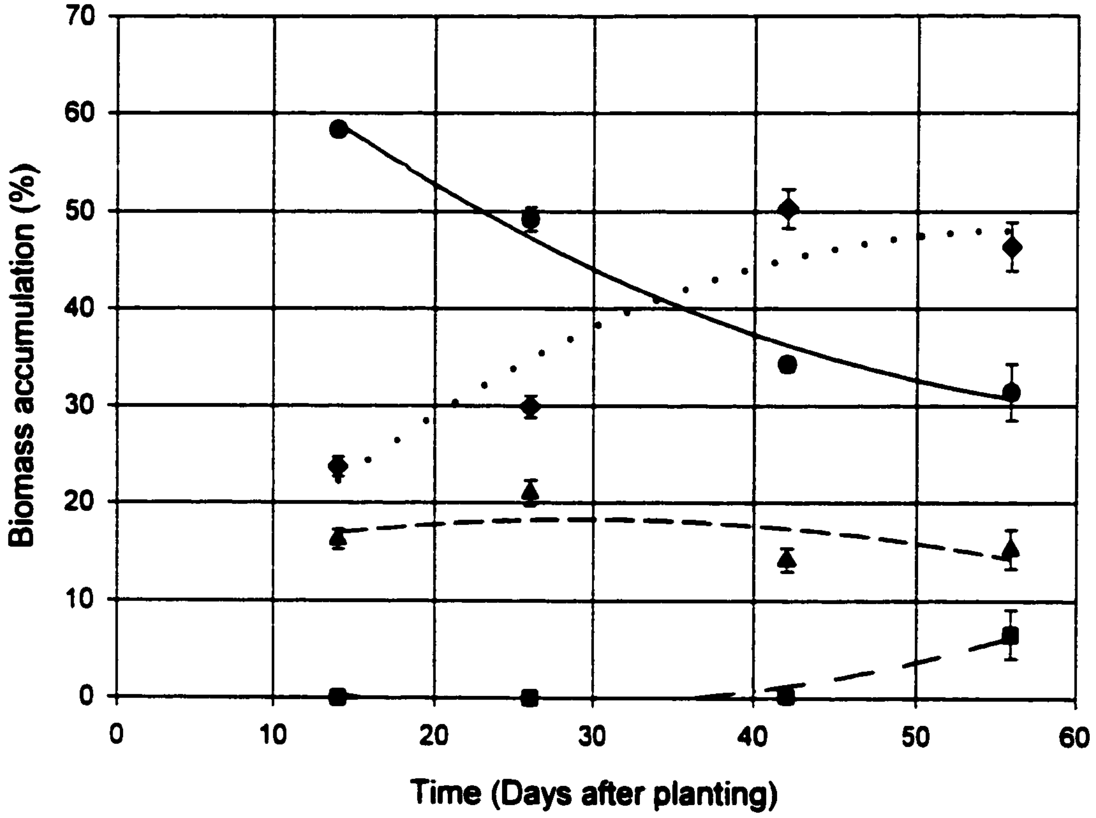

Figure 2.43. Percent of total biomass accumulation of Wyoming-Platte County brown seeded proso millet biotype. Leaf (— $y = 76.0949 - 1.3660*DAP + 0.0099*DAP^2$, $r^2 = 0.85$), stem (····· $y = 2.2648 + 1.6289*DAP - 0.0145*DAP^2$, $r^2 = 0.76$), root (– – $y = 13.4987 + 0.3301*DAP - 0.0056*DAP^2$, $r^2 = 0.04^{ns}$), and reproductive tissue (- · - $y = 4.4272 - 0.4098*DAP + 0.0079*DAP^2$, $r^2 = 0.38$).

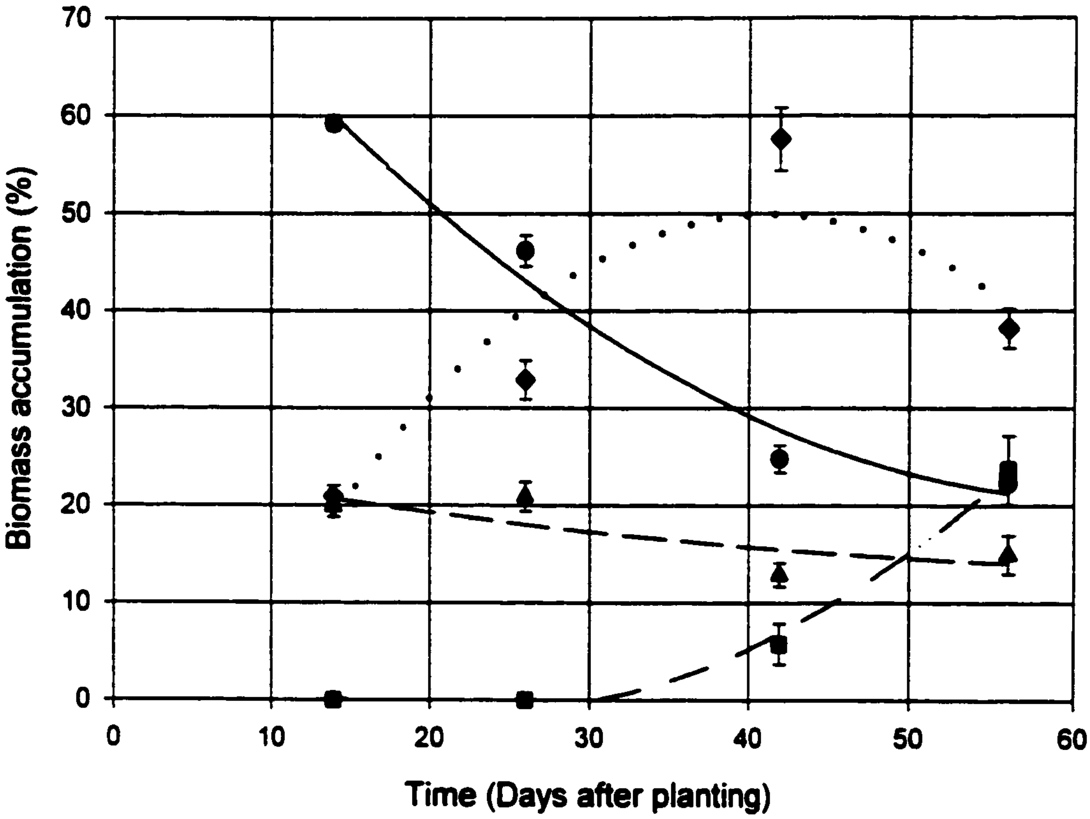

Figure 2.44. Percent of total biomass accumulation of Colorado-Weld County black seeded proso millet biotype. Leaf (— y = 85.8391 - 2.0730*DAP + 0.0164*DAP^2, r^2 = 0.92), stem (····· y = --21.2572 + 3.4584*DAP - 0.0420*DAP^2, r^2 = 0.70), root (- - y = 24.3838 - 0.2934*DAP + 0.0020*DAP^2, r^2 = 0.21), and reproductive tissue (- · - y = 10.0957 - 1.0101*DAP + 0.0222*DAP^2, r^2 = 0.73).

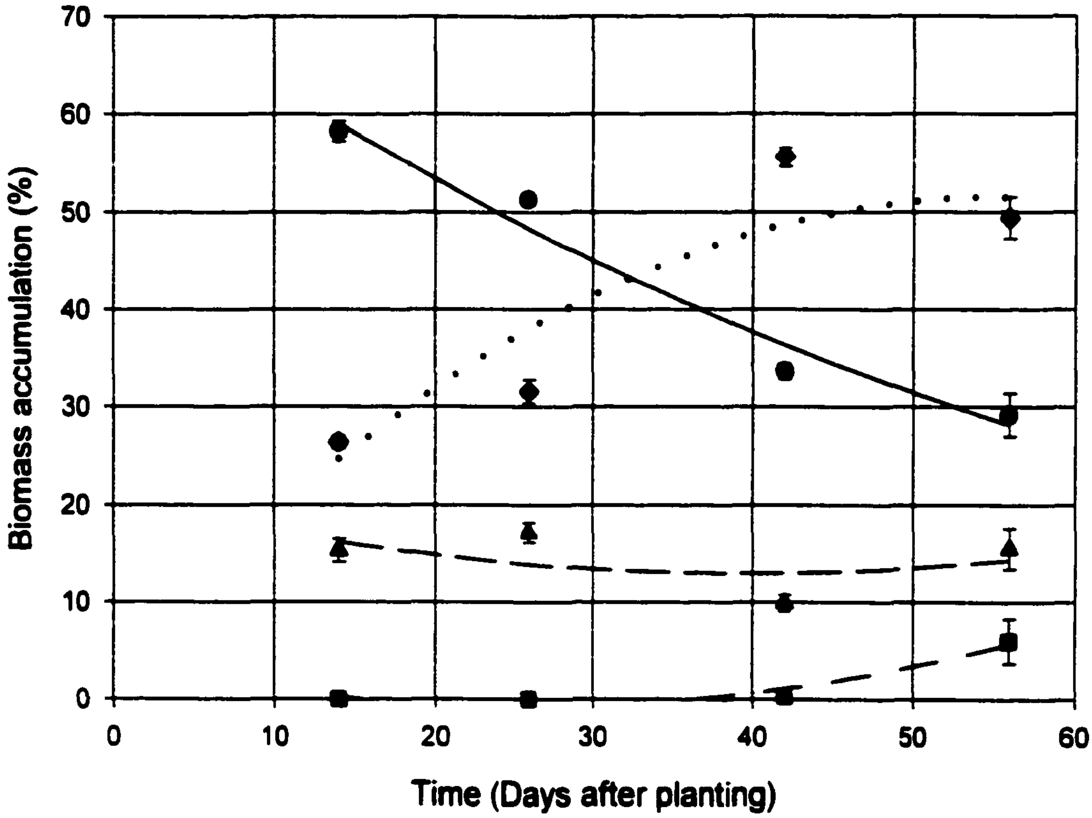

Figure 2.45. Percent of total biomass accumulation of Colorado-Weld County tan seeded proso millet biotype. Leaf (— $y = 73.6527 - 1.1189*DAP + 0.0055*DAP^2$, $r^2 = 0.897$), stem (····· $y = 3.3462 + 1.7437*DAP - 0.0158*DAP^2$, $r^2 = 0.78$), root (- - $y = 20.7927 - 0.3970*DAP + 0.0050*DAP^2$, $r^2 = 0.01^{ns}$), and reproductive tissue (- · - $y = 4.0139 - 0.3716*DAP + 0.0072*DAP^2$, $r^2 = 0.35$).

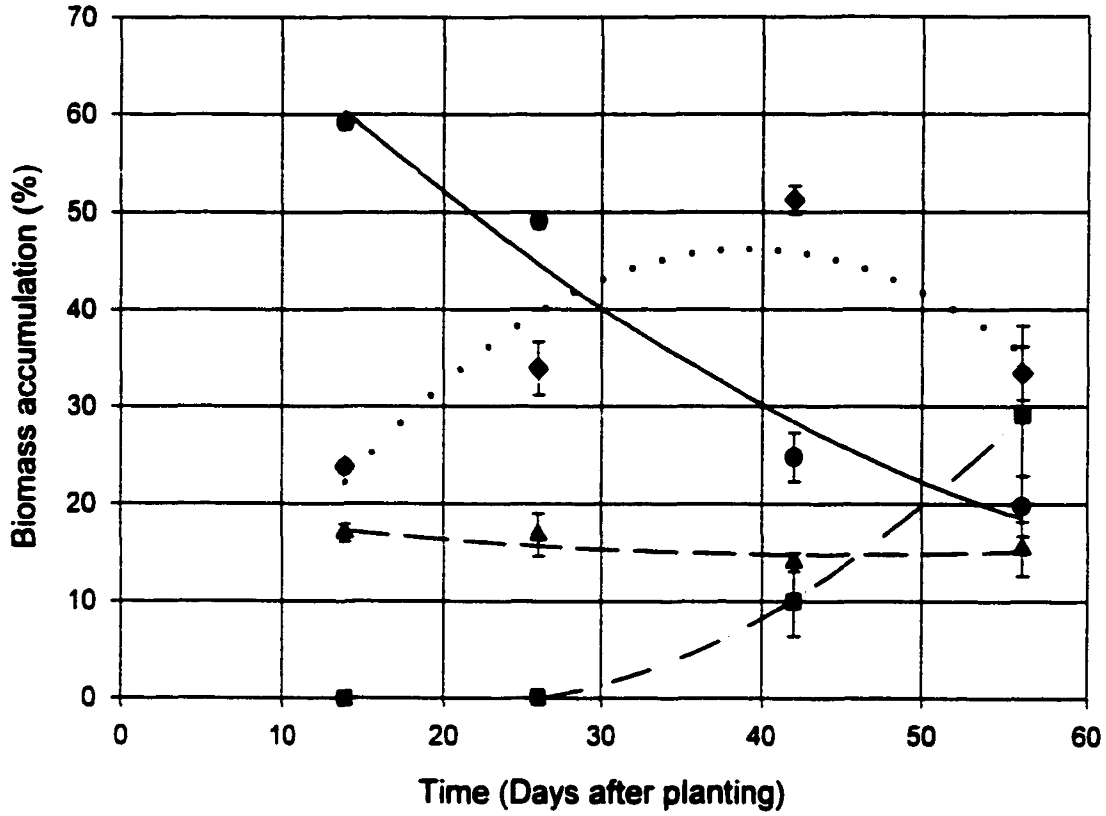

Figure 2.46. Percent of total biomass accumulation of South Dakota brown seeded proso millet biotype. Leaf (— $y = 82.3169 - 1.7164*DAP + 0.0103*DAP^2$, $r^2 = 0.88$), stem (····· $y = -12.0599 + 2.9815*DAP - 0.0381*DAP^2$, $r^2 = 0.65$), root (- - $y = 19.9975 - 0.2367*DAP + 0.0027*DAP^2$, $r^2 = 0.01^{ns}$), and reproductive tissue (- · - $y = 8.8450 - 0.9572*DAP + 0.0236*DAP^2$, $r^2 = 0.48$).

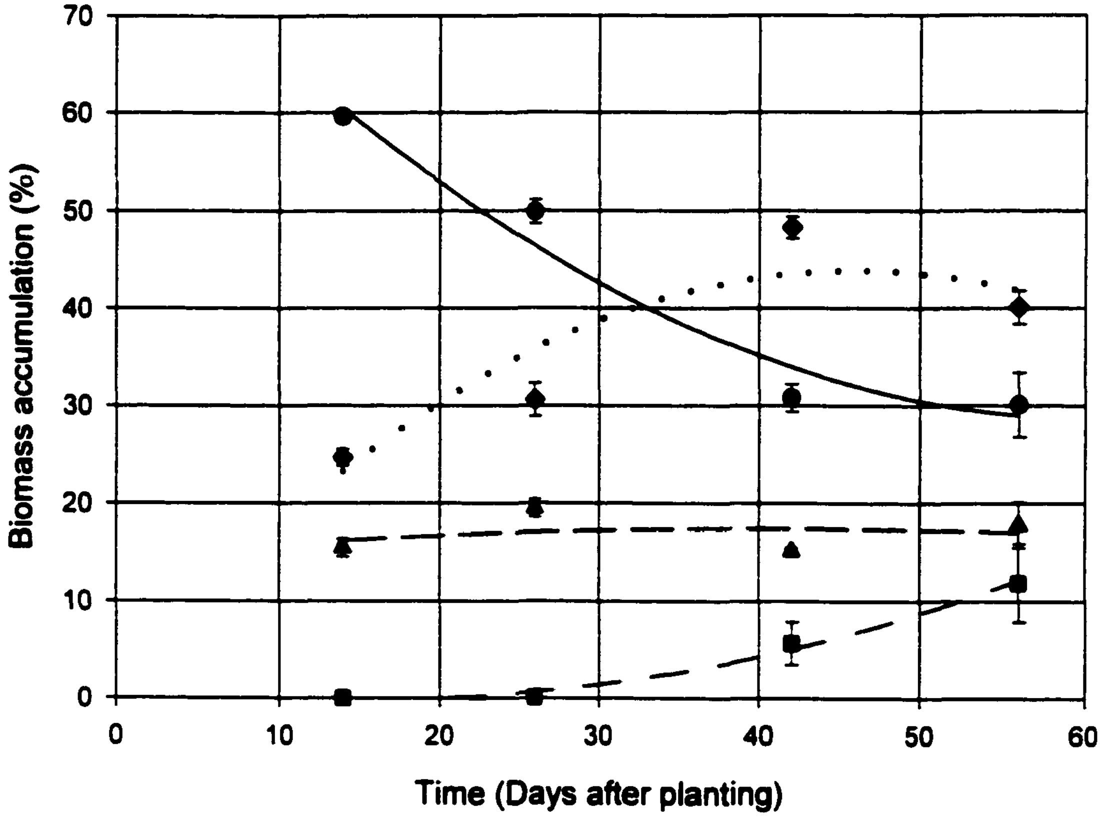

Figure 2.47. Percent of total biomass accumulation of Colorado white seeded proso millet biotype. Leaf (— $y = 82.0524 - 1.7374*DAP + 0.0141*DAP^2$, $r^2 = 0.84$), stem (····· $y = 1.3663 + 1.8473*DAP - 0.0201*DAP^2$, $r^2 = 0.71$), root (– – $y = 14.6280 + 0.1395*DAP - 0.0017*DAP^2$, $r^2 = 0.01^{ns}$), and reproductive tissue (– · – $y = 1.5950 - 0.2314*DAP + 0.0075*DAP^2$, $r^2 = 0.38$).

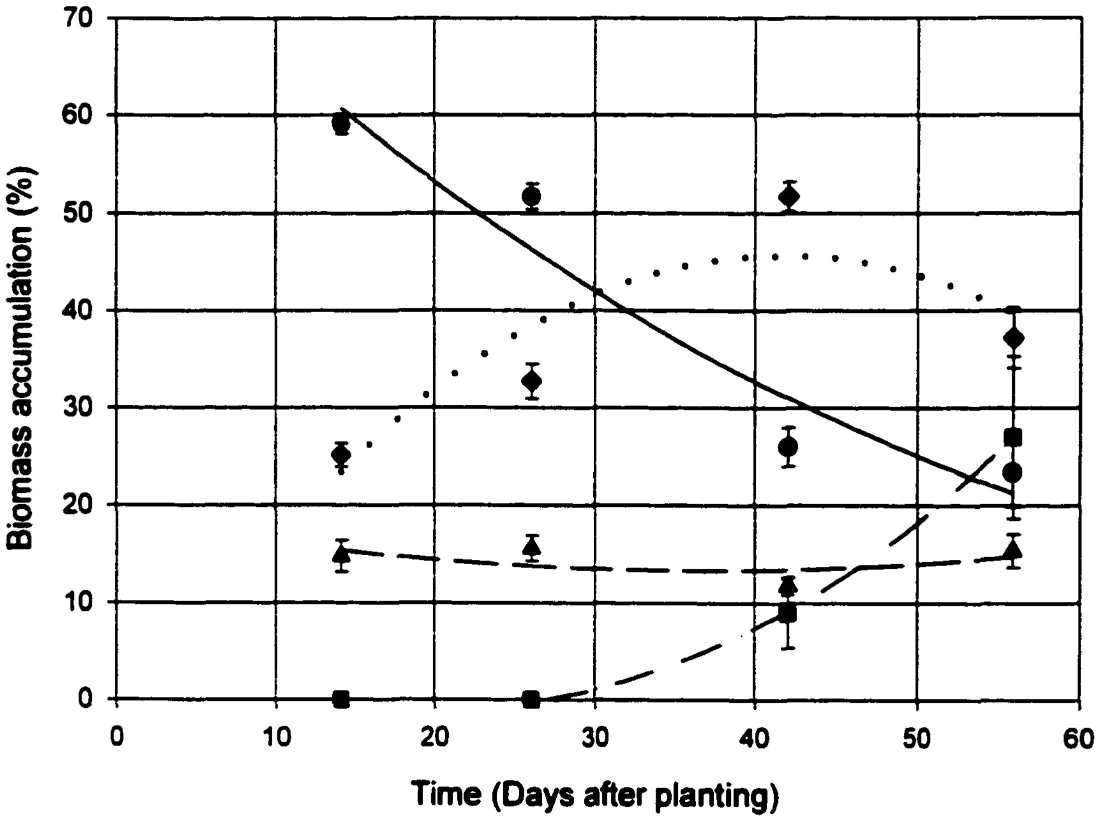

Figure 2.48. Percent of total biomass accumulation of Colorado orange seeded proso millet biotype. Leaf (— $y = 80.7637 - 1.5556*DAP + 0.0088*DAP^2$, $r^2 = 0.84$), stem (····· $y = -4.7803 + 2.4162*DAP - 0.0290*DAP^2$, $r^2 = 0.63$), root (- - $y = 18.6595 - 0.2899*DAP + 0.0039*DAP^2$, $r^2 = 0.01^{ns}$), and reproductive tissue (- · - $y = 8.5970 - 0.9197*DAP + 0.0222*DAP^2$, $r^2 = 0.48$).

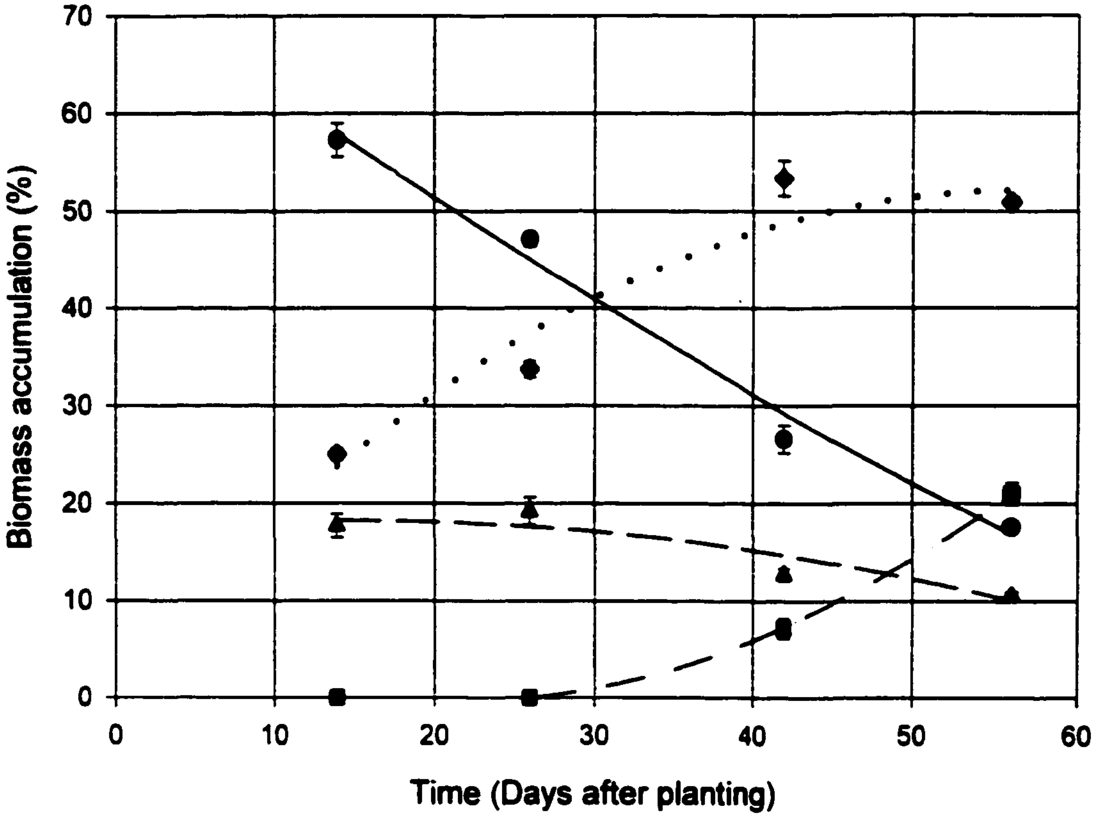

Figure 2.49. Percent of total biomass accumulation of Colorado-Larimer white seeded proso millet biotype. Leaf (— $y = 74.4639 - 1.2263*DAP + 0.0035*DAP^2$, $r^2 = 0.87$), stem (····· $y = -2.0229 + 1.7659*DAP - 0.0156*DAP^2$, $r^2 = 0.81$), root (- - $y = 17.1387 + 0.1454*DAP - 0.0049*DAP^2$, $r^2 = 0.32$), and reproductive tissue (- · - $y = 6.3045 - 0.6850*DAP + 0.0169*DAP^2$, $r^2 = 0.49$).

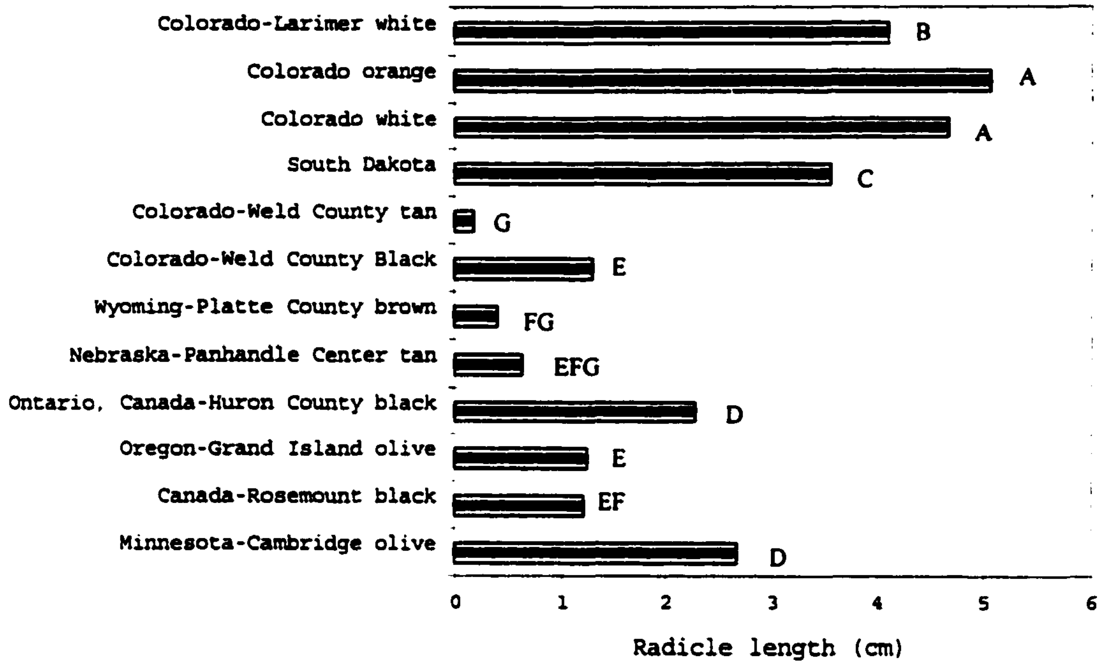

Figure 2.50. Radicle length of domestic and wild proso millet biotypes measured 5 days after incubation. Bars followed by the same letter are not significantly different ($p<0.05$.).

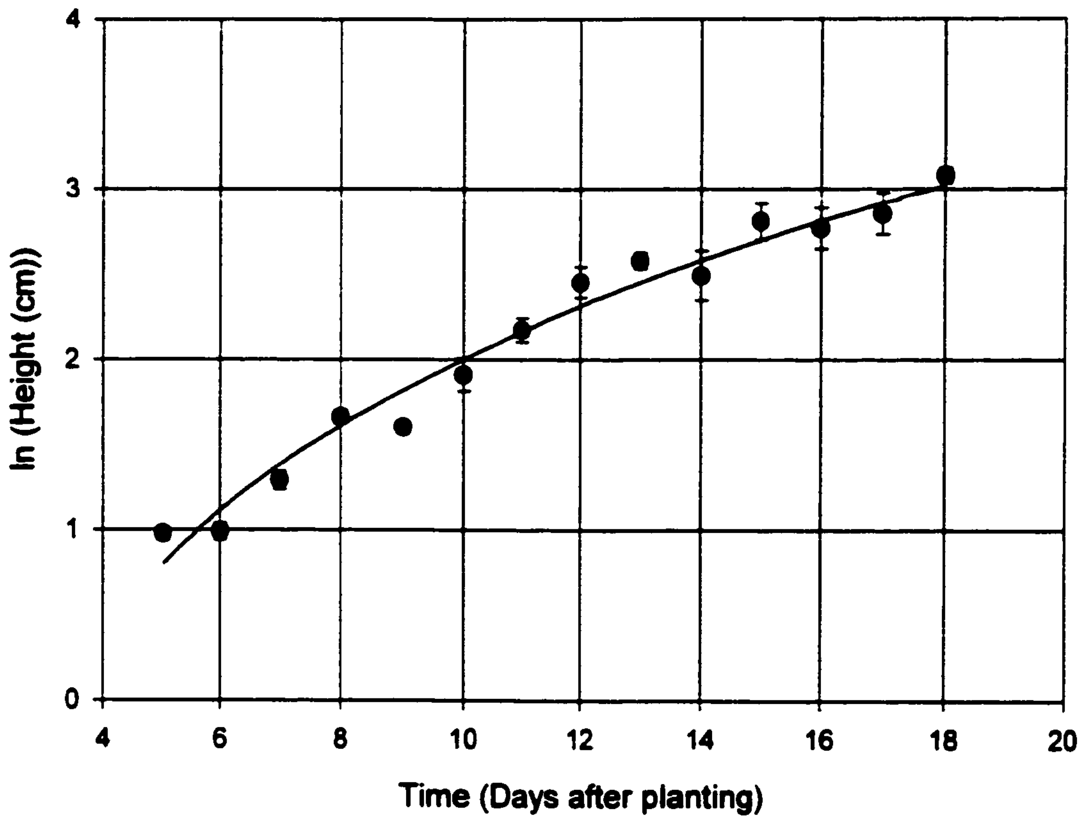

Figure 2.51. Height of Minnesota-Cambridge olive seeded proso millet biotype 5 to 18 days after planting. Points with vertical bars represent means with standard error of the mean. Curve corresponds to the equation Y = -1.9911 + 1.7334*ln(DAP) r^2 = 0.92.

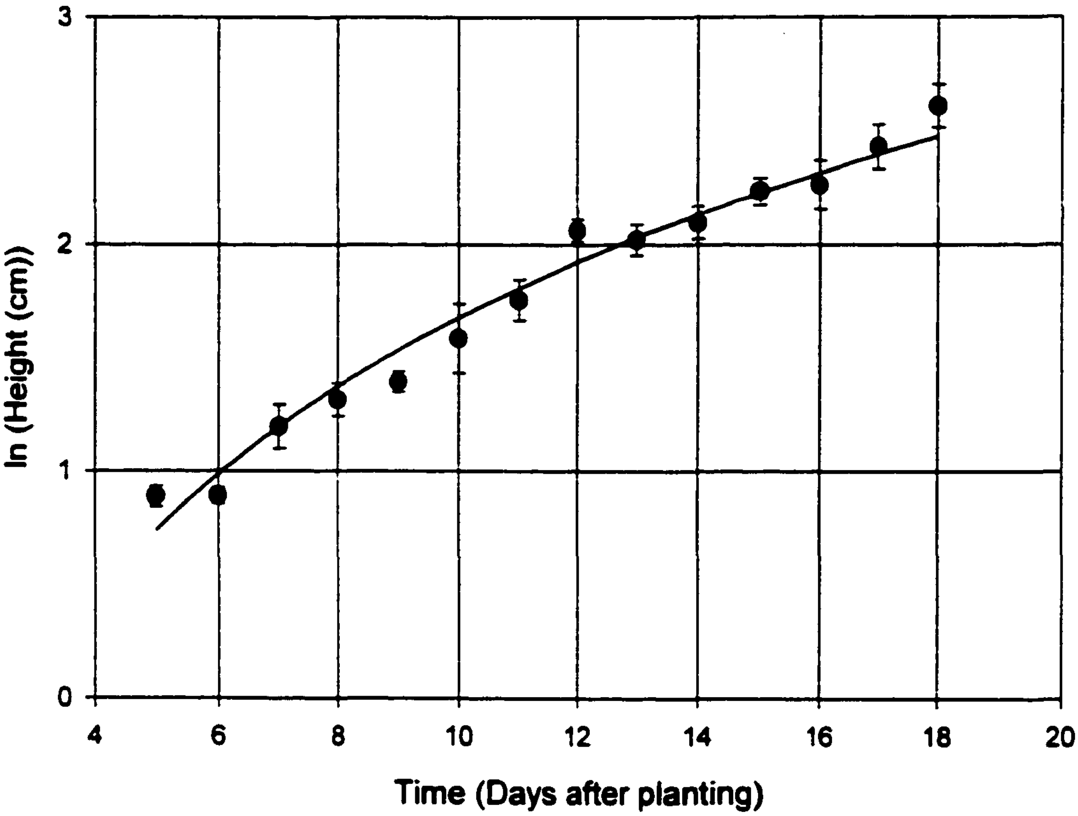

Figure 2.52. Height of Canada-Rosemount black seeded proso millet biotype 5 to 18 days after planting. Points with vertical bars represent means with standard error of the mean. Curve corresponds to the equation $Y = -1.4357 + 1.3514*\ln(DAP)$ $r^2 = 0.89$.

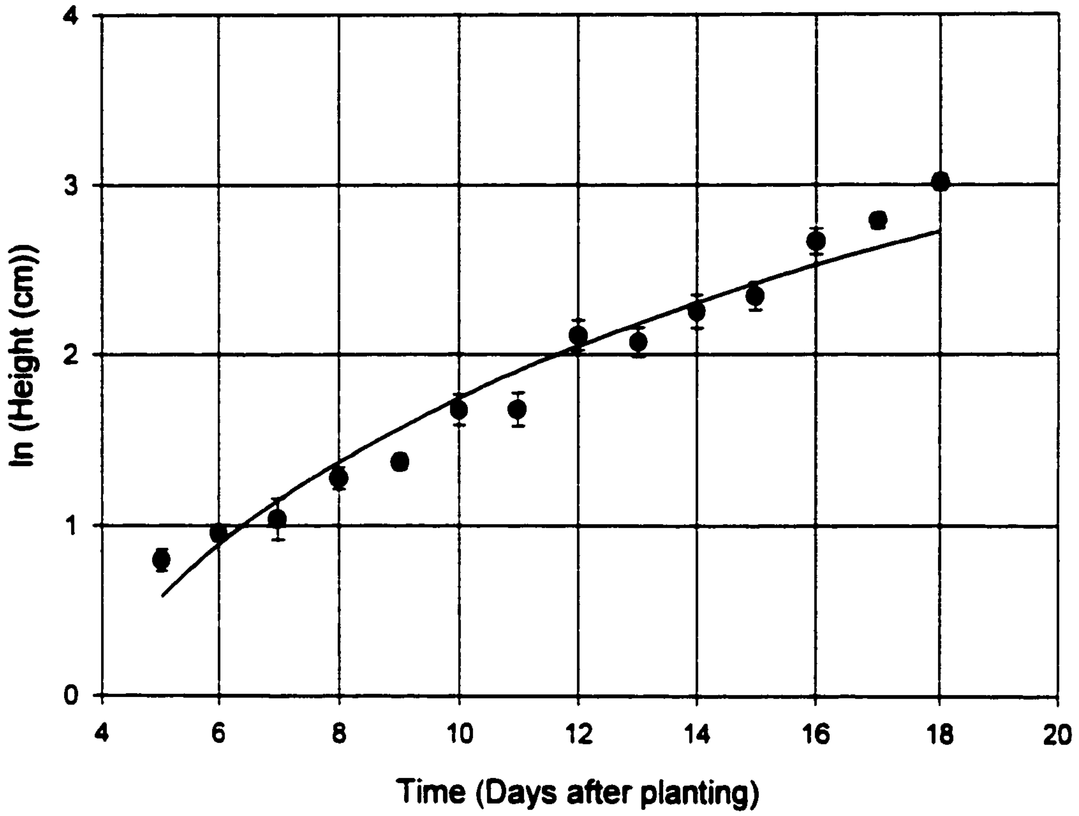

Figure 2.53. Height of Oregon-Grand Island olive seeded proso millet biotype 5 to 18 days after planting. Points with bars represent means with standard error of the mean. Curve corresponds to the equation Y = -2.1031 + 1.6709*ln(DAP) r^2 = 0.90.

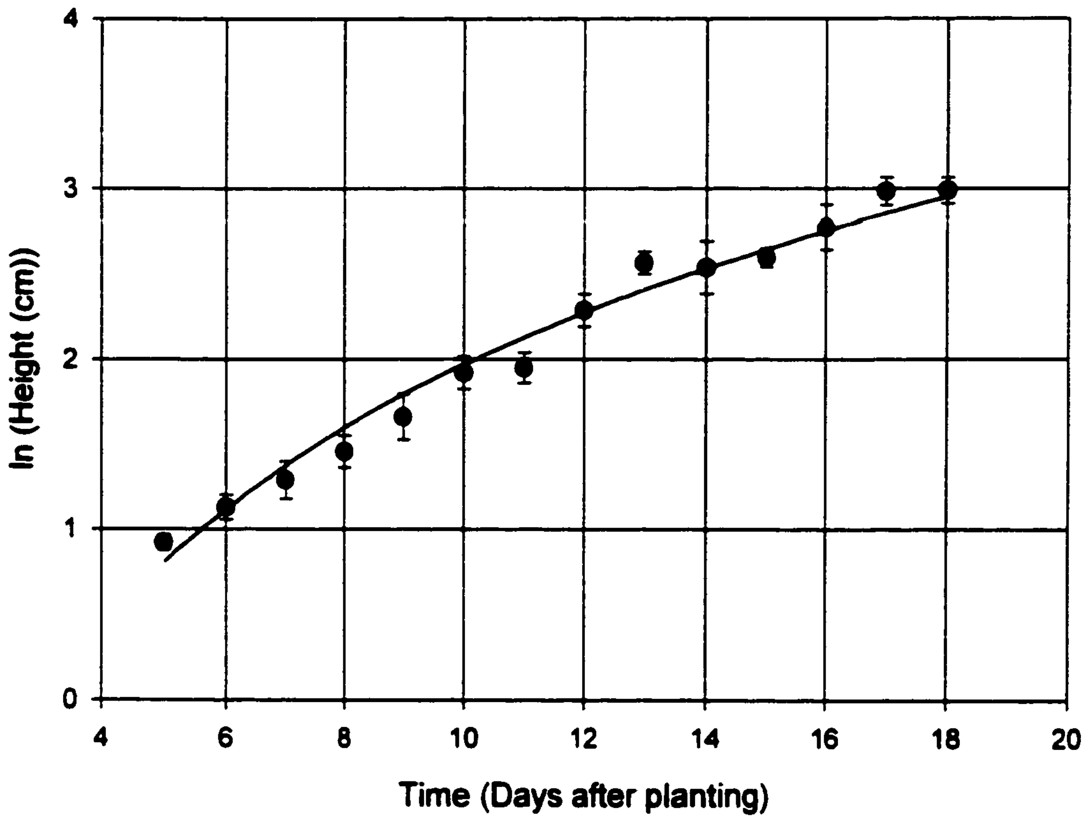

Figure 2.54. Height of Ontario, Canada-Huron County black seeded proso millet biotype 5 to 18 days after planting. Points with bars represent means with the standard error of the mean. Curve corresponds to the equation Y = -1.8877 + 1.6744*ln(DAP) r^2 = 0.90.

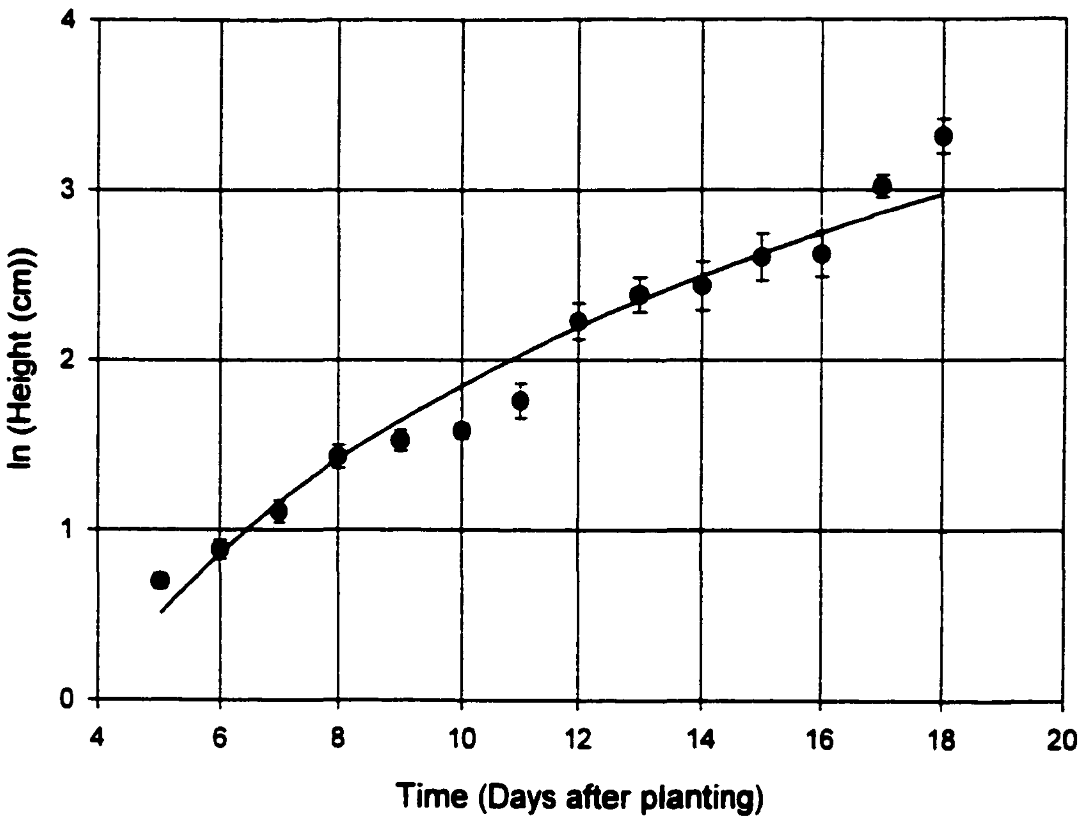

Figure 2.55. Height of Nebraska-Panhandle Center tan seeded proso millet biotype 5 to 18 days after planting. Points with bars represent means with standard error of the mean. Curve corresponds to the equation Y = -2.5836 + 1.9229*ln(DAP) r^2 = 0.90.

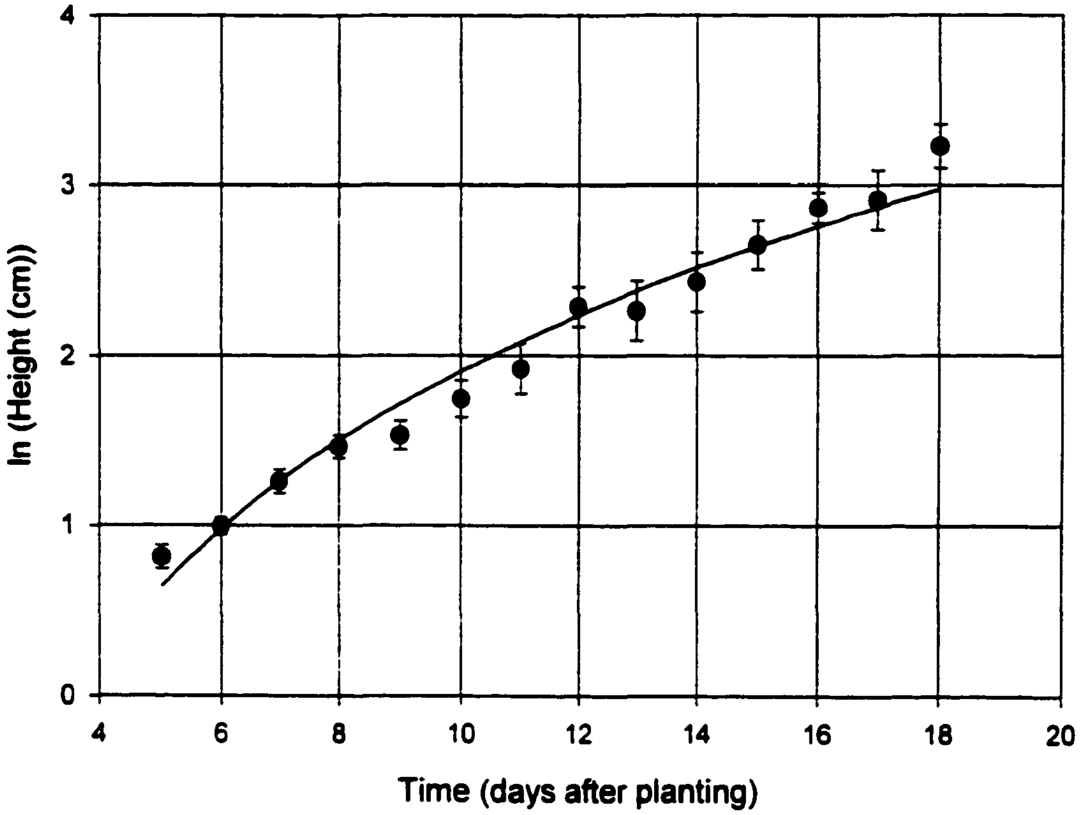

Figure 2.56. Height of Wyoming-Platte County brown seeded proso millet biotype 5 to 18 days after planting. Points with bars represent means with standard error of the mean. Curve corresponds to the equation $Y = -2.2827 + 1.8186*\ln(DAP)$ $r^2 = 0.86$.

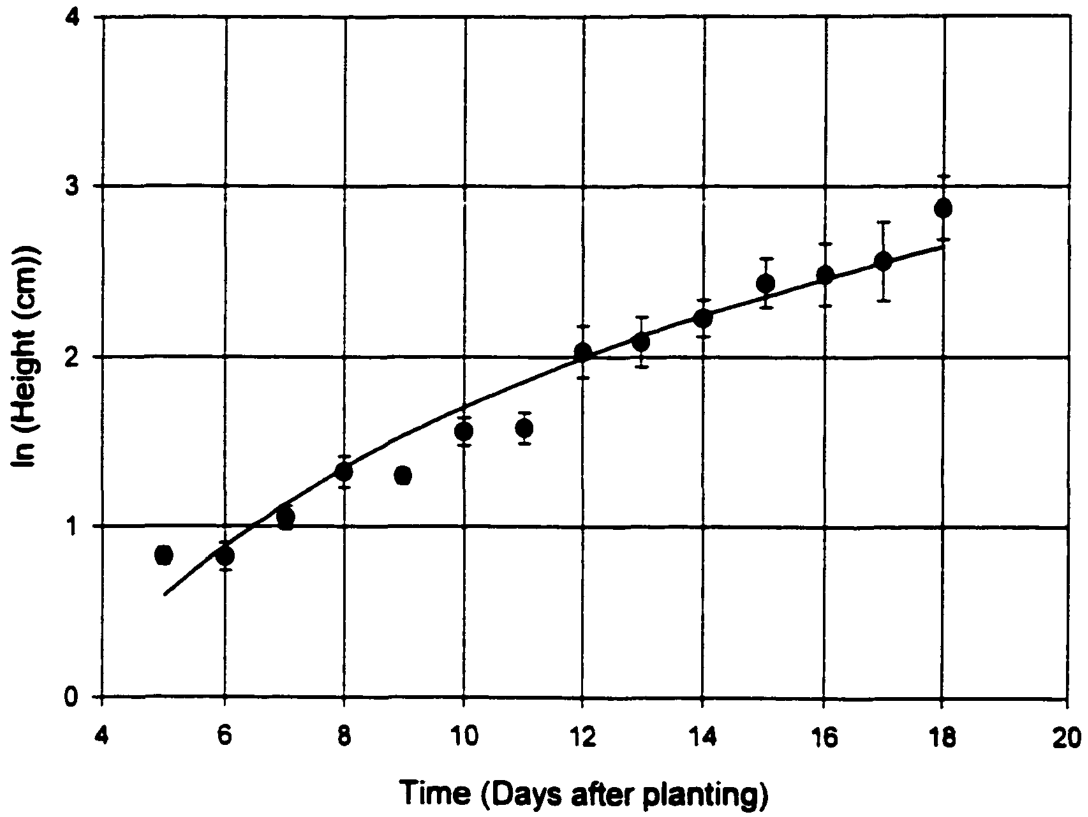

Figure 2.57. Height of Colorado-Weld County black seeded proso millet biotype 5 to 18 days after planting. Points with bars represent means with standard error of the mean. Curve corresponds to the equation Y = -1.9949 + 1.6061*ln(DAP) r^2 = 0.81.

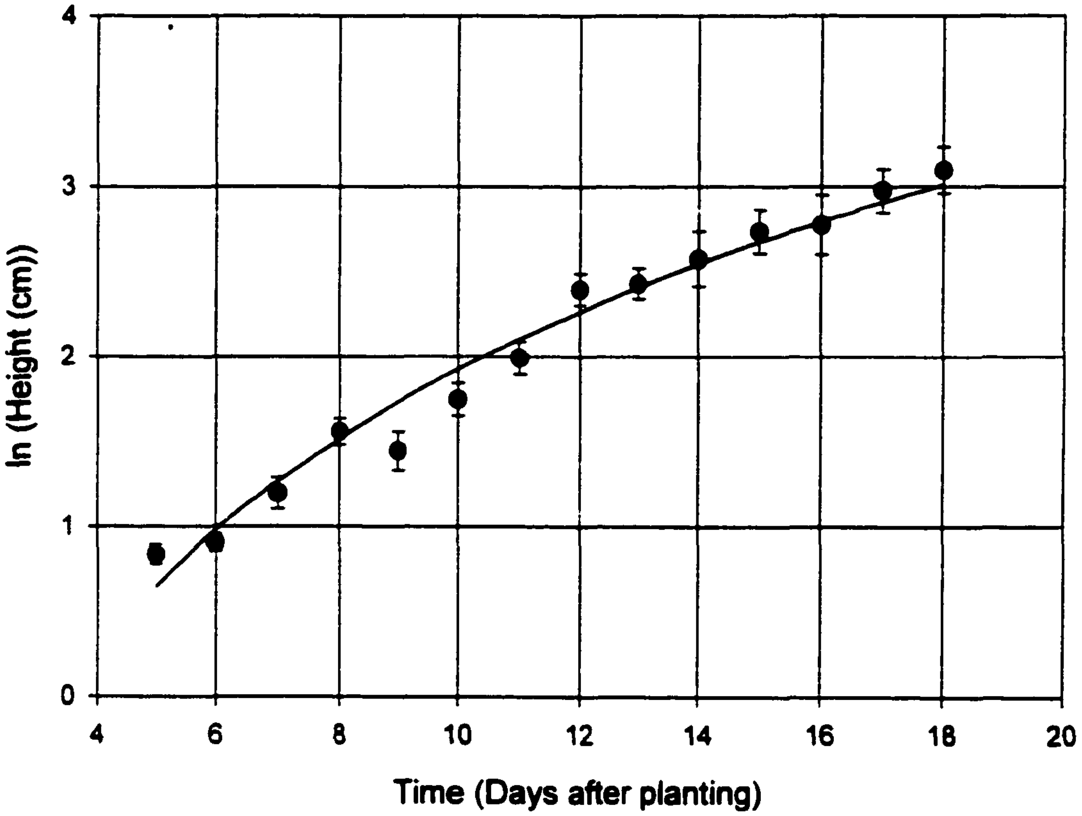

Figure 2.58. Height of Colorado-Weld County tan seeded proso millet biotype 5 to 18 days after planting. Points with bars represent means with standard error of the mean. Curve corresponds to the equation $Y = -2.3301 + 1.8481*\ln(DAP)$ $r^2 = 0.89$.

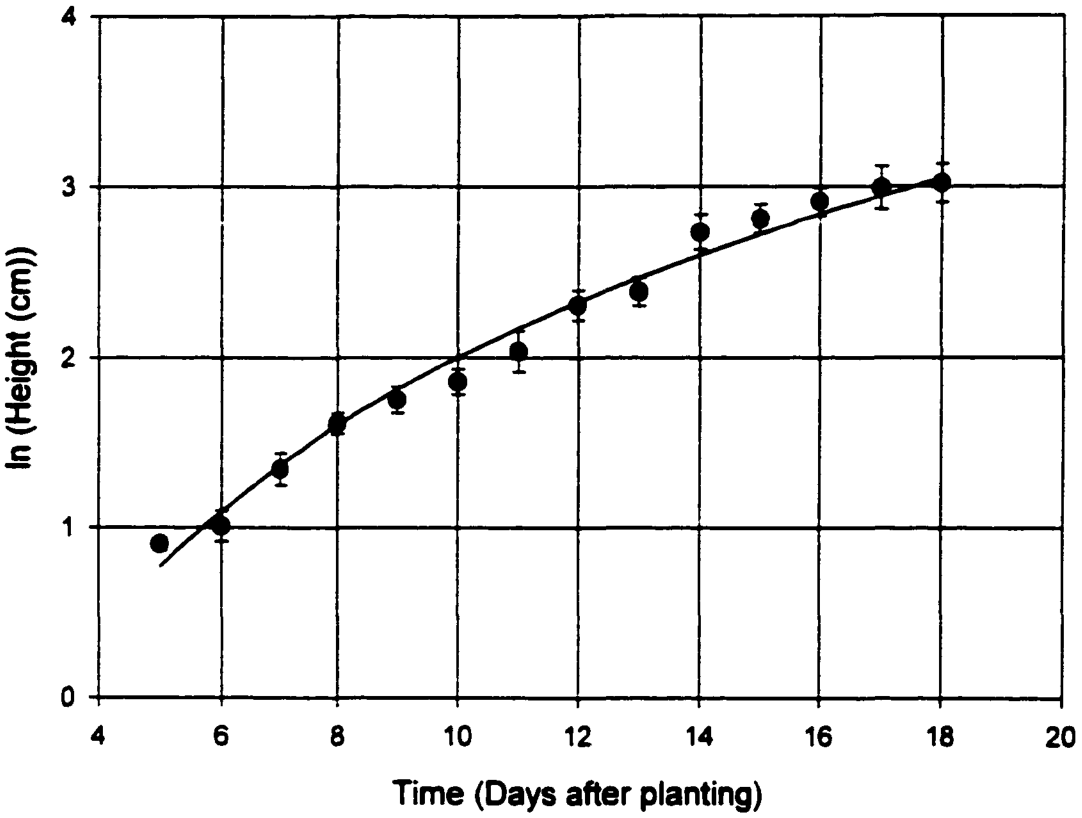

Figure 2.59. Height of South Dakota brown olive seeded proso millet biotype 5 to 18 days after planting. Points with bars represent means with standard error of the mean. Curve corresponds to the equation Y = -2.0932 + 1.7781*ln(DAP) r^2 = 0.92.

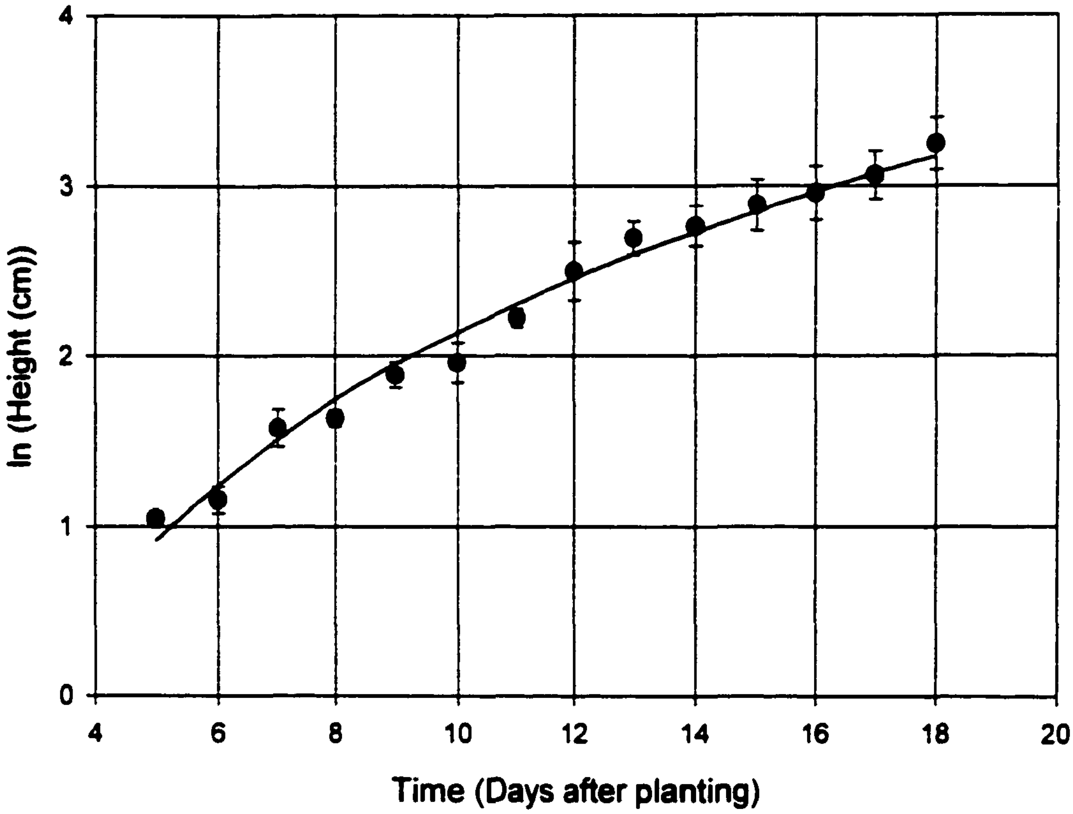

Figure 2.60. Height of Colorado white seeded proso millet biotype 5 to 18 days after planting. Points with bars represent means with standard error of the mean. Curve corresponds to the equation Y = -2.0932 + 1.7781*ln(DAP) r^2 = 0.92.

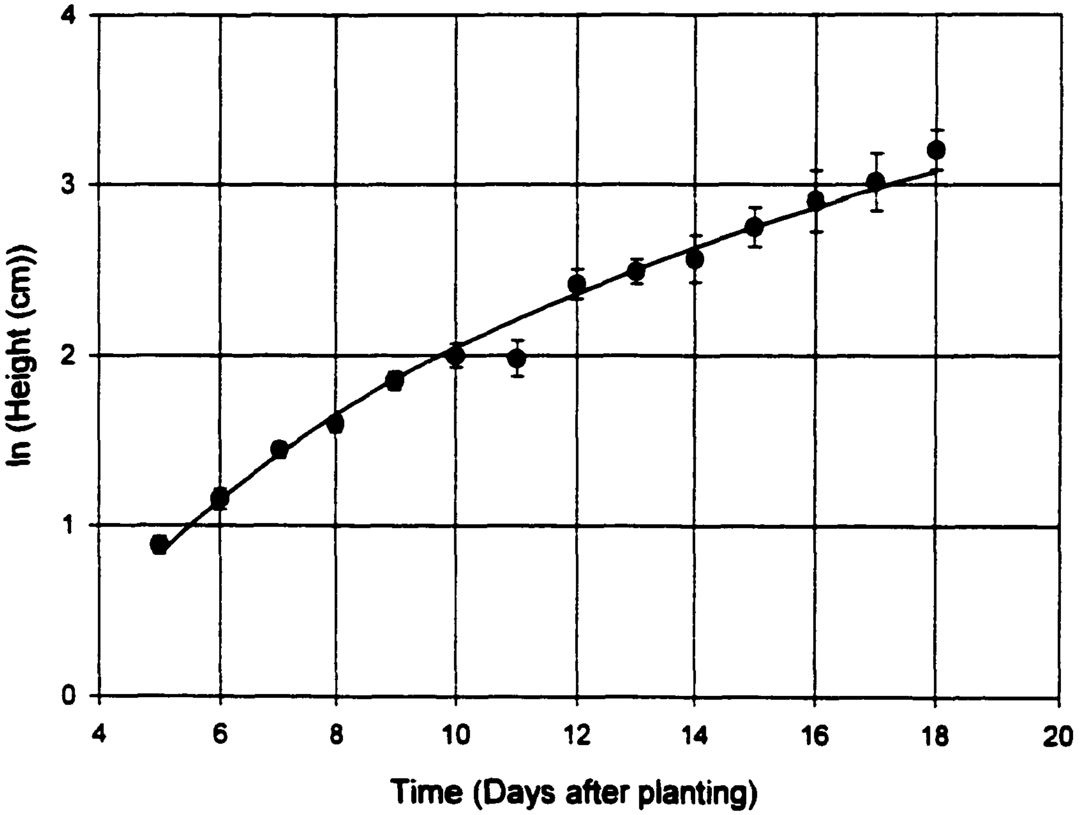

Figure 2.61. Height of Colorado orange seeded proso millet biotype 5 to 18 days after planting. Points with bars represent means with standard error of the mean. Curve corresponds to the equation Y = -1.9915 + 1.7538*ln(DAP) r^2 = 0.91.

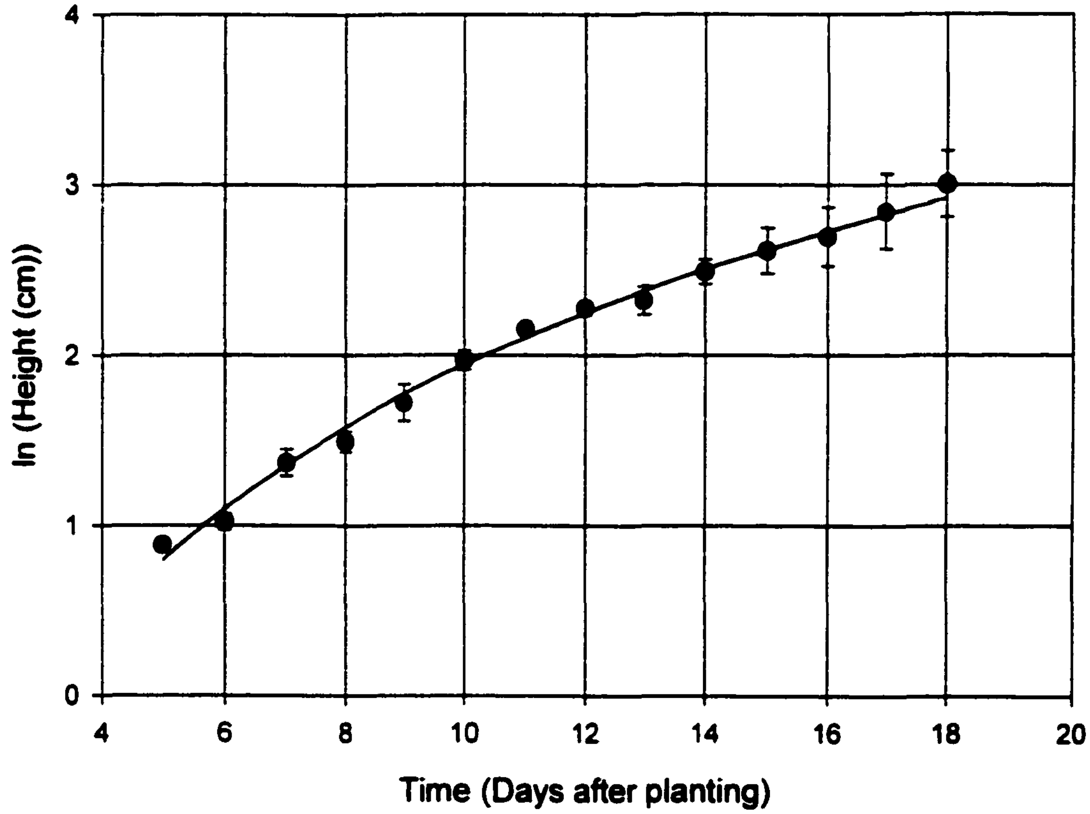

Figure 2.62. Height of Colorado-Larimer white seeded proso millet biotype 5 to 18 days after planting. Points with bars represent means with standard error of the mean. Curve corresponds to the equation Y = -1.8755 + 1.6597*ln(DAP) r^2 = 0.92.

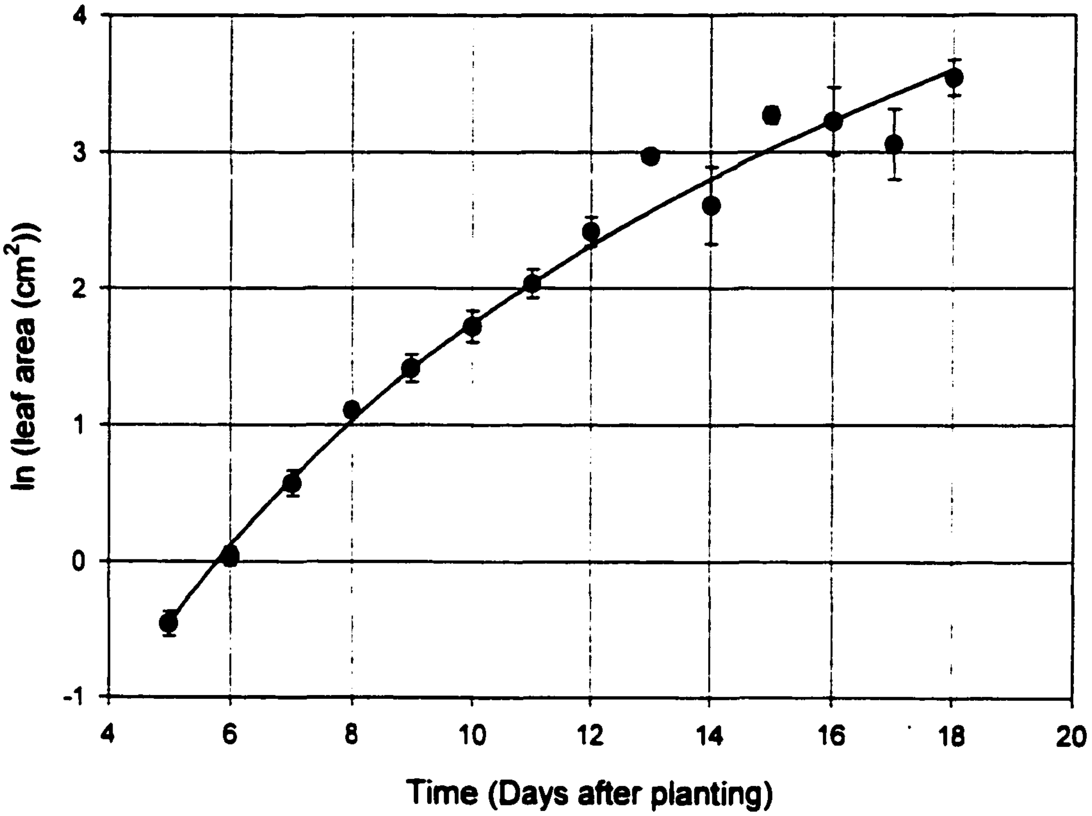

Figure 2.63. Leaf area of Minnesota-Cambridge olive seeded proso millet biotype 5 to 18 days after planting. Points with vertical bars represent means with standard error of the mean. Curve corresponds to the equation Y = -5.5635 + 3.1712*ln(DAP) r^2 = 0.93.

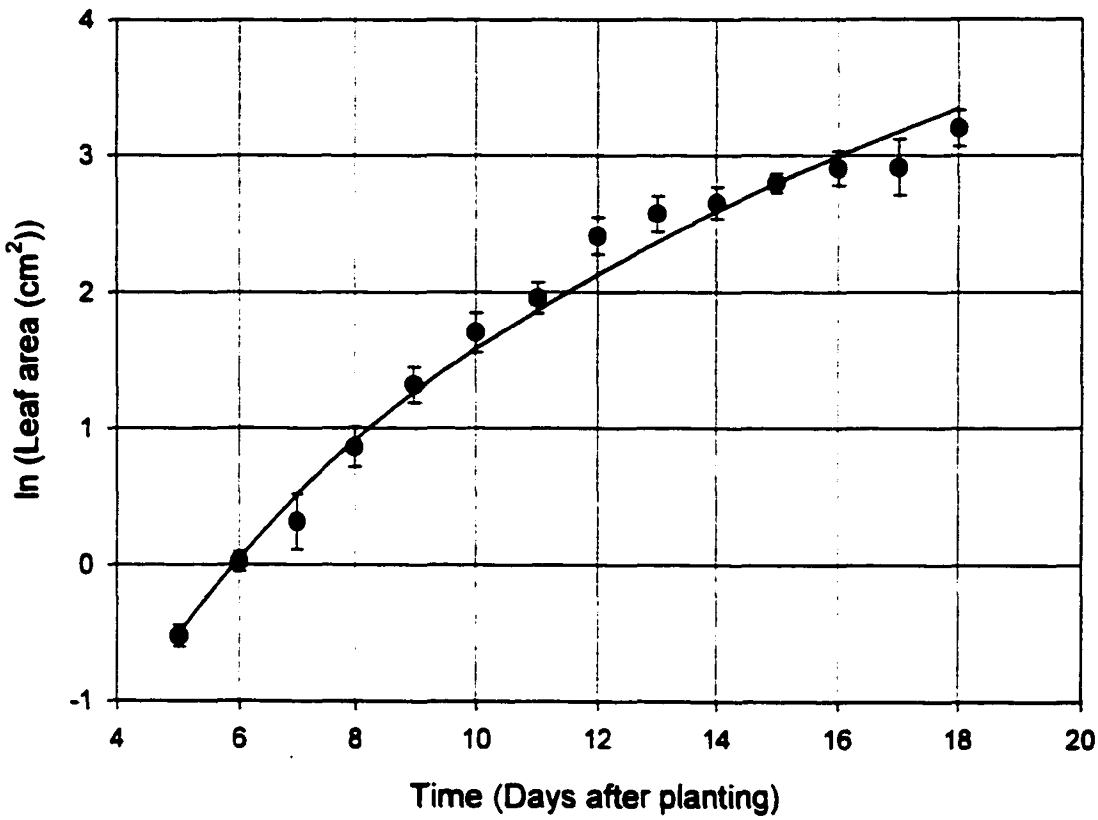

Figure 2.64. Leaf area of Canada-Rosemount black seeded proso millet biotype 5 to 18 days after planting. Points with vertical bars represent means with standard error of the mean. Curve corresponds to the equation $Y = -5.3318 + 3.0019*\ln(DAP)$ $r^2 = 0.94$.

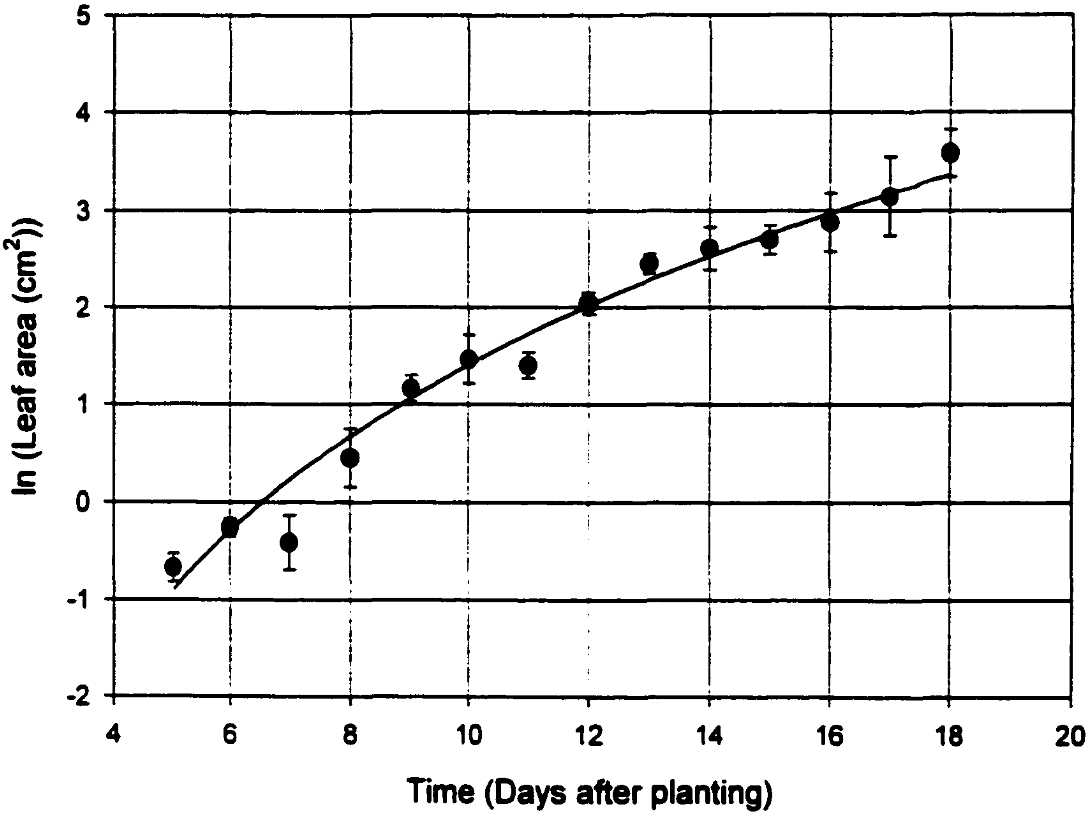

Figure 2.65. Leaf area of Oregon-Grand Island olive seeded proso millet biotype 5 to 18 days after planting. Points with bars represent means with standard error of the mean. Curve plotted is correspond to the equation Y = -6.2431 + 3.3240*ln(DAP) r^2 = 0.90.

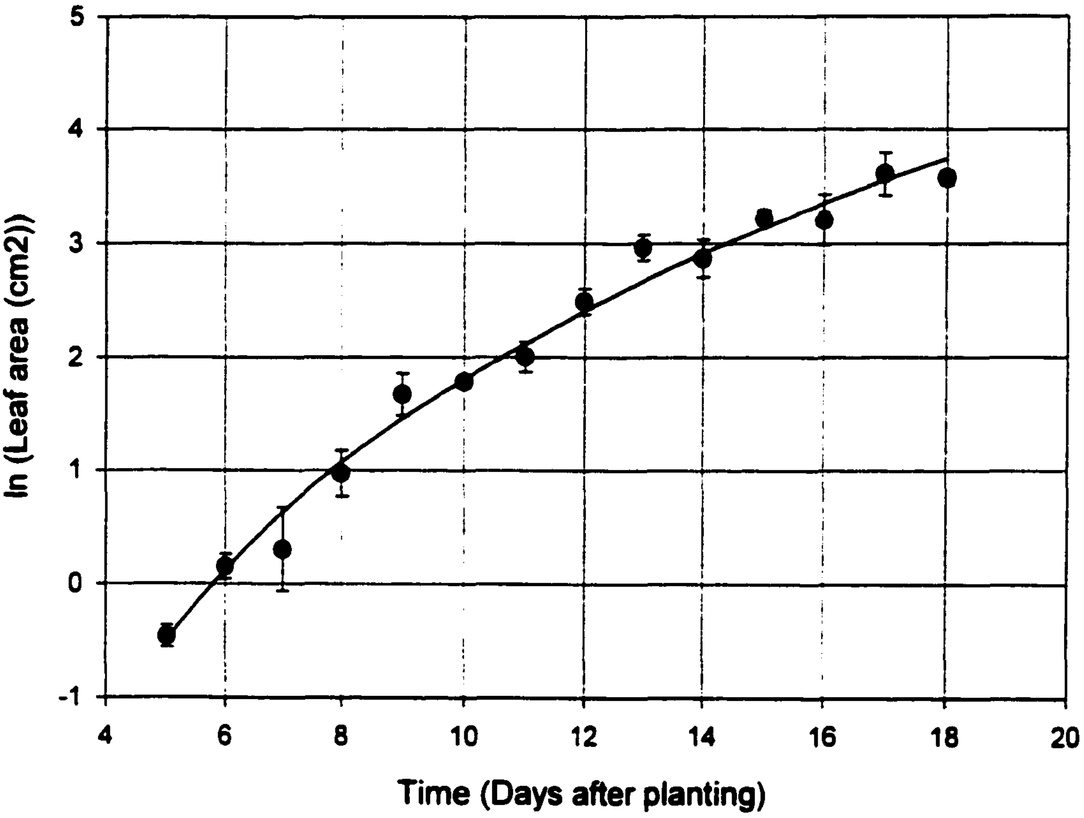

Figure 2.66. Leaf area of Ontario, Canada-Huron County black seeded proso millet biotype 5 to 18 days after planting. Points with bars represent means with the standard error of the mean. Curve corresponds to the equation Y = -5.7830 + 3.2955*ln(DAP) r^2 = 0.90.

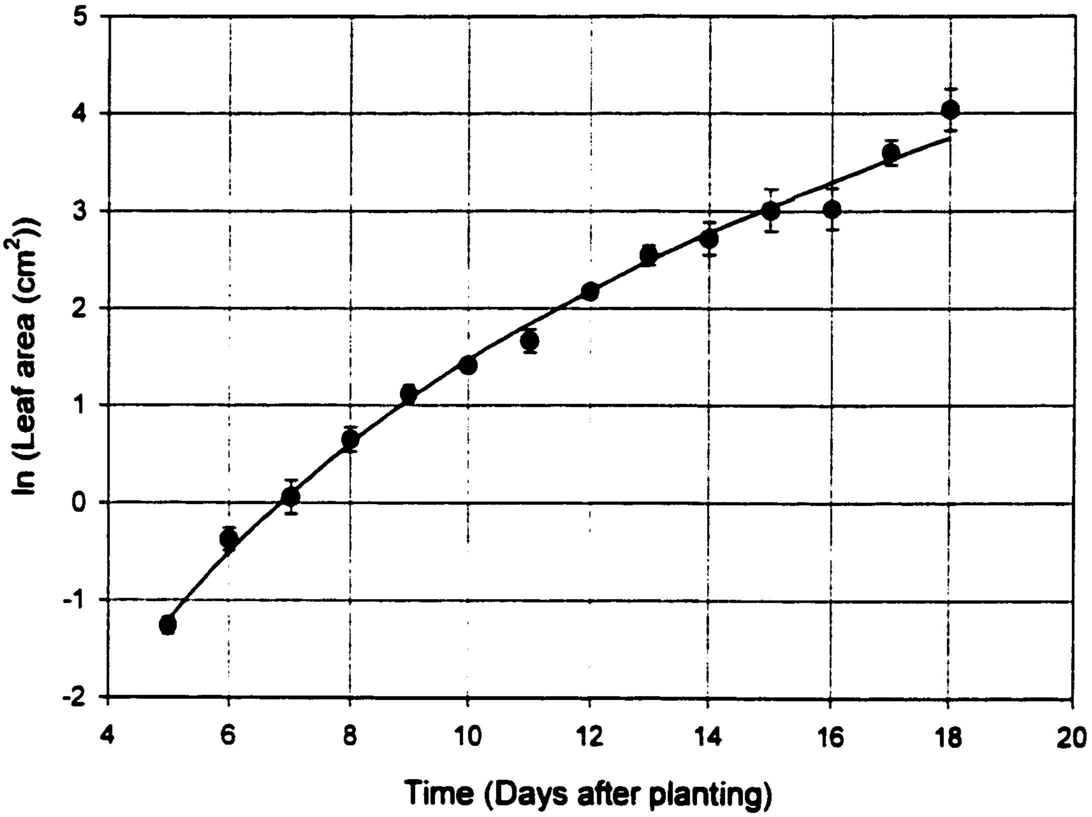

Figure 2.67. Leaf area of Nebraska-Panhandle Center tan seeded proso millet biotype 5 to 18 days after planting. Points with bars represent means with standard error of the mean. Curve corresponds to the equation $Y = -7.4403 + 3.8709*\ln(DAP)$ $r^2 = 0.95$.

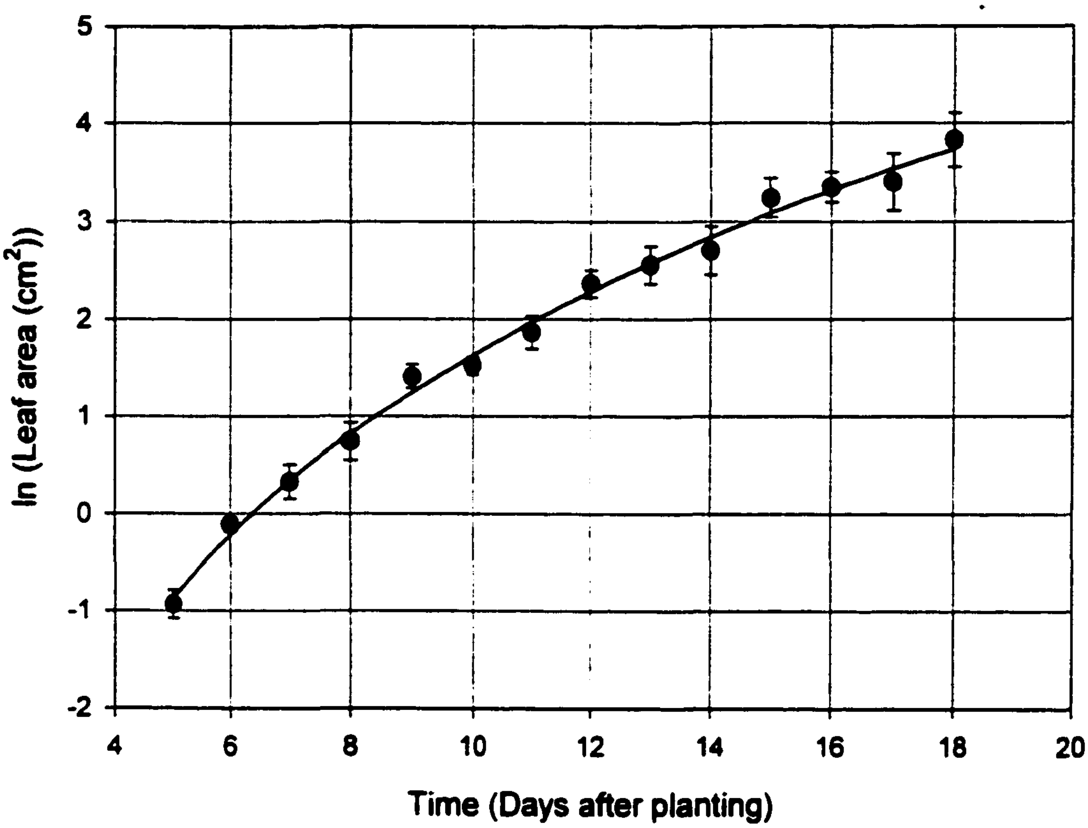

Figure 2.68. Leaf area of Wyoming-Platte County brown seeded proso millet biotype 5 to 18 days after planting. Points with bars represent means with standard error of the mean. Curve corresponds to the equation $Y = -6.6691 + 3.6020*\ln(DAP)$ $r^2 = 0.92$.

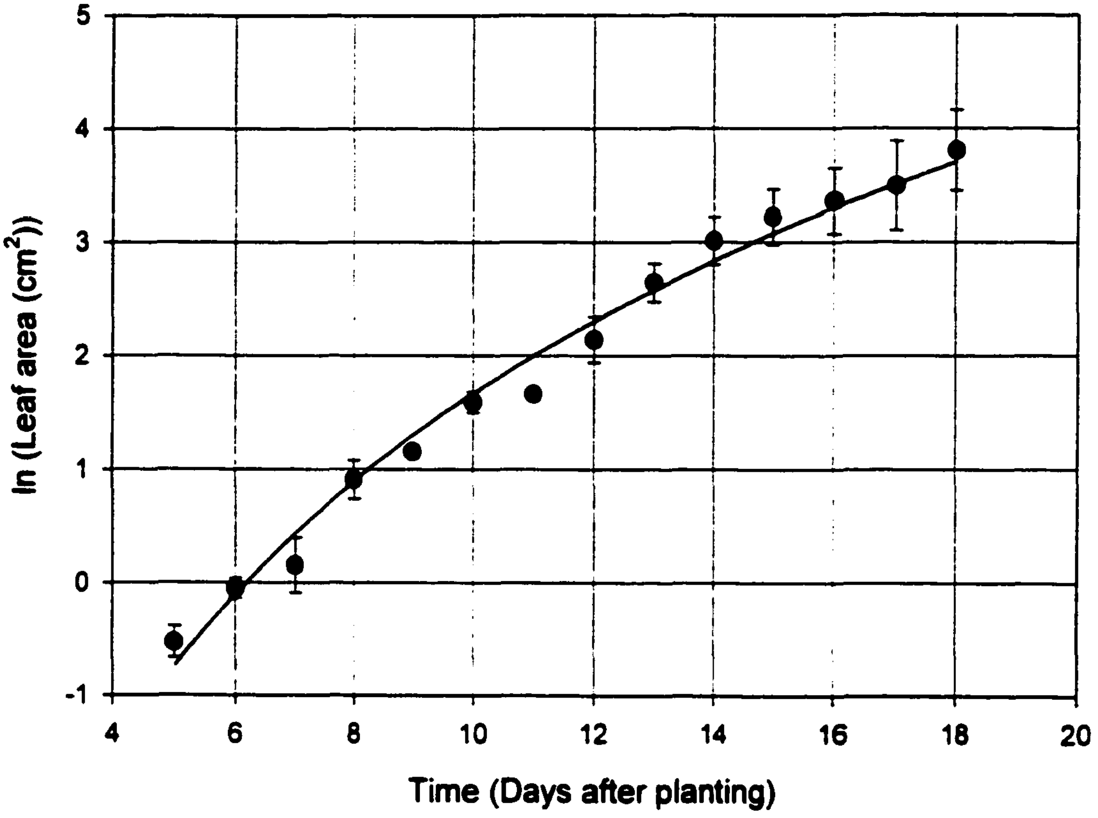

Figure 2.69. Leaf area of Colorado-Weld County black seeded proso millet biotype 5 to 18 days after planting. Points with bars represent means with standard error of the mean. Curve corresponds to the equation $Y = -6.3226 + 3.4693*\ln(DAP)$ $r^2 = 0.89$.

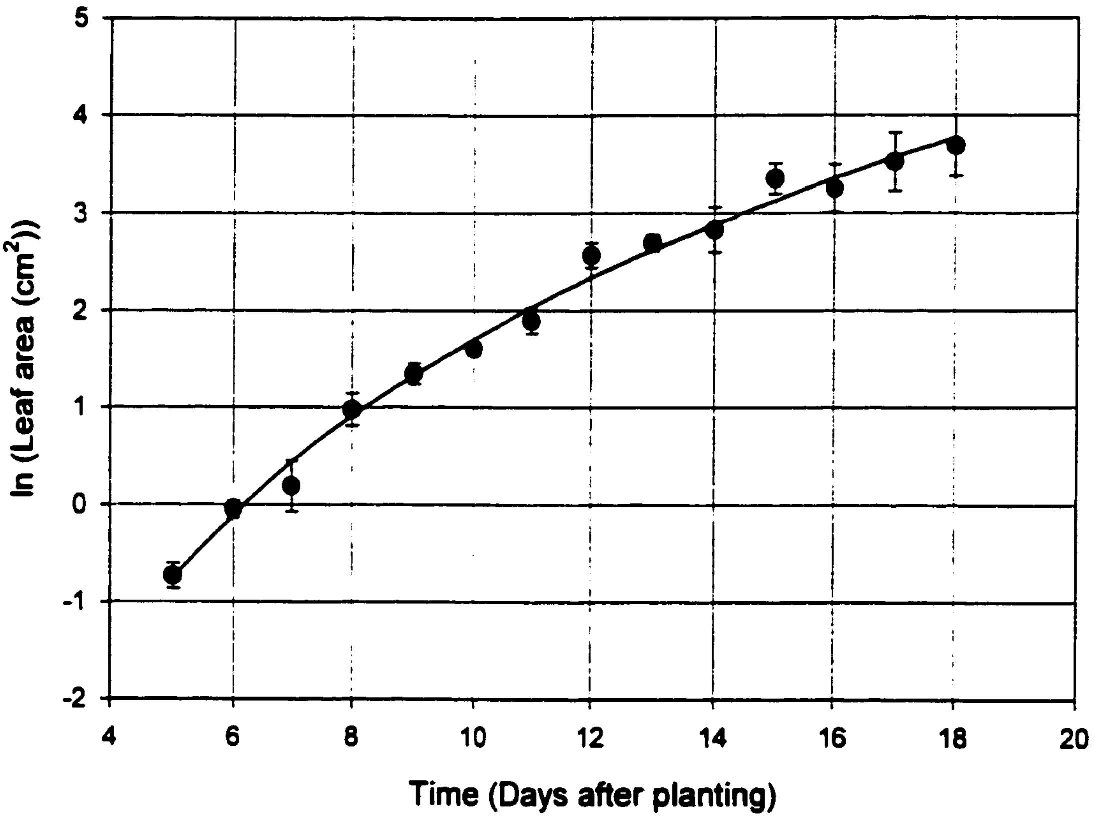

Figure 2.70. Leaf area of Colorado-Weld County tan seeded proso millet biotype 5 to 18 days after planting. Points with bars represent means with standard error of the mean. Curve corresponds to the equation Y = -6.4266 + 3.5291*ln(DAP) r^2 = 0.92.

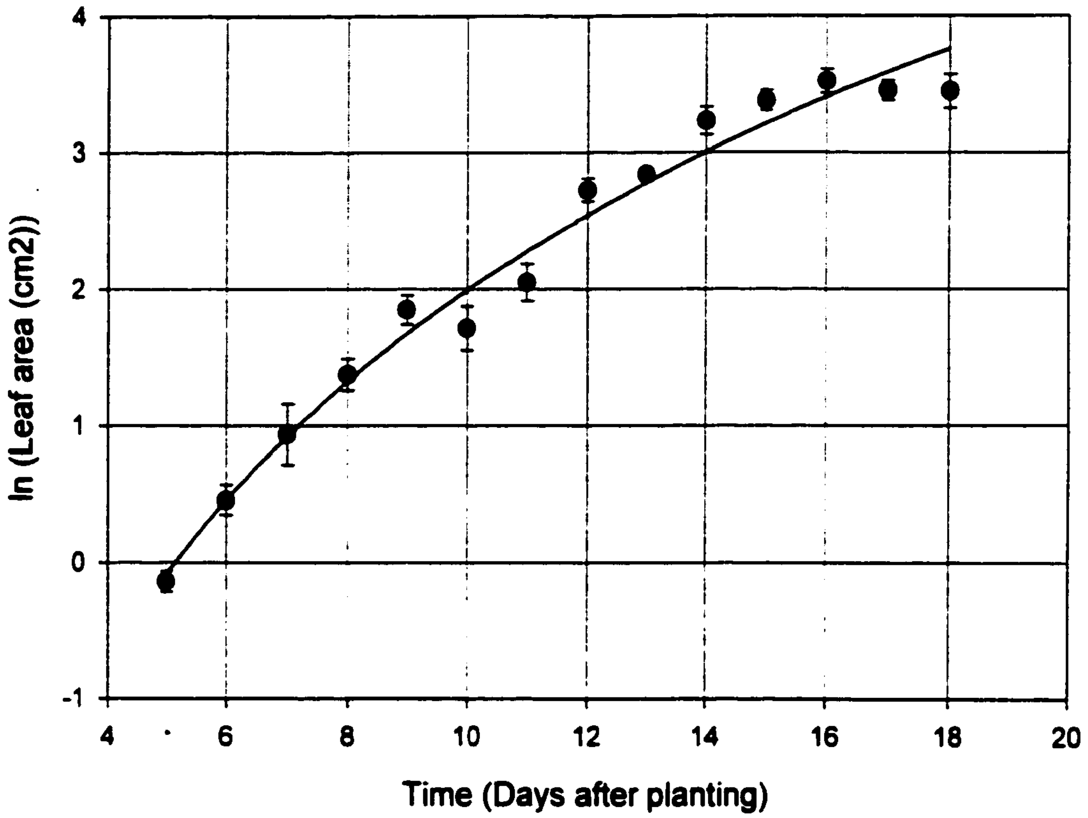

Figure 2.71. Leaf area of South Dakota brown olive seeded proso millet biotype 5 to 18 days after planting. Points with bars represent means with standard error of the mean. Curve corresponds to the equation Y = -4.9213 + 3.0021*ln(DAP) $r^2 = 0.94$.

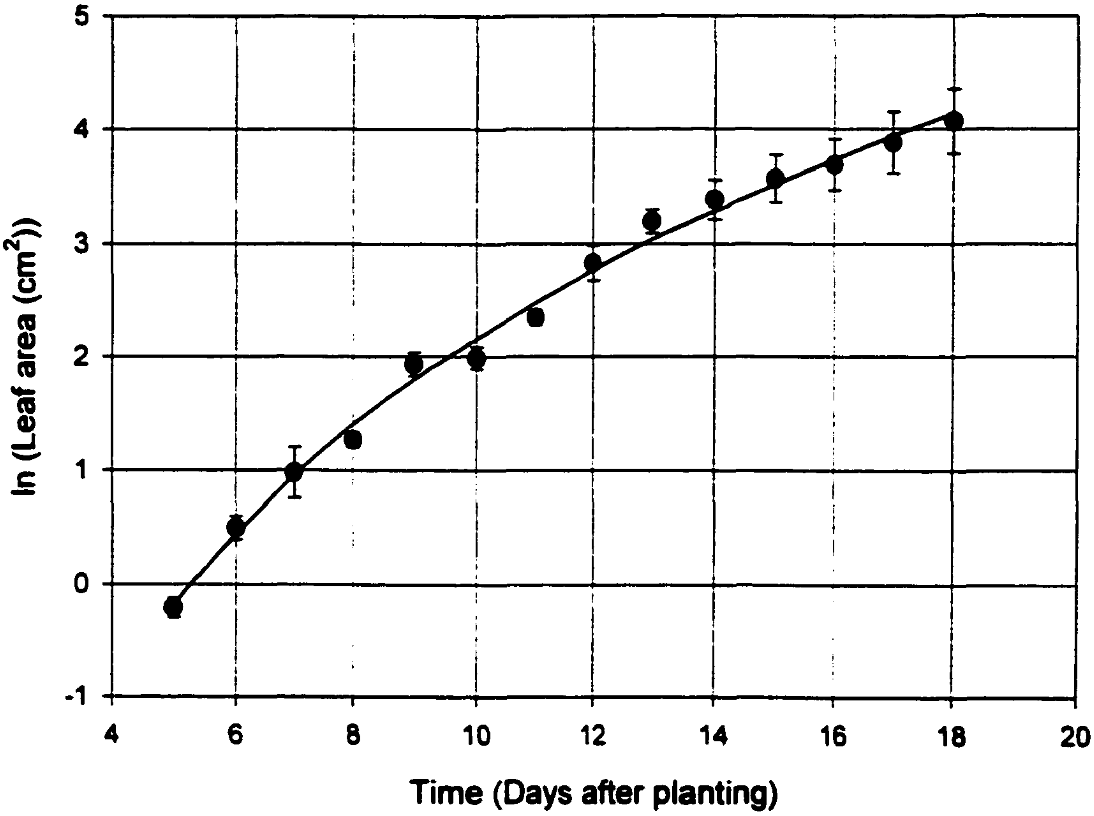

Figure 2.72. Leaf area of Colorado white seeded proso millet biotype 5 to 18 days after planting. Points with bars represent means with standard error of the mean. Curve corresponds to the equation Y = -5.5876 + 3.3621*ln(DAP) r^2 = 0.93.

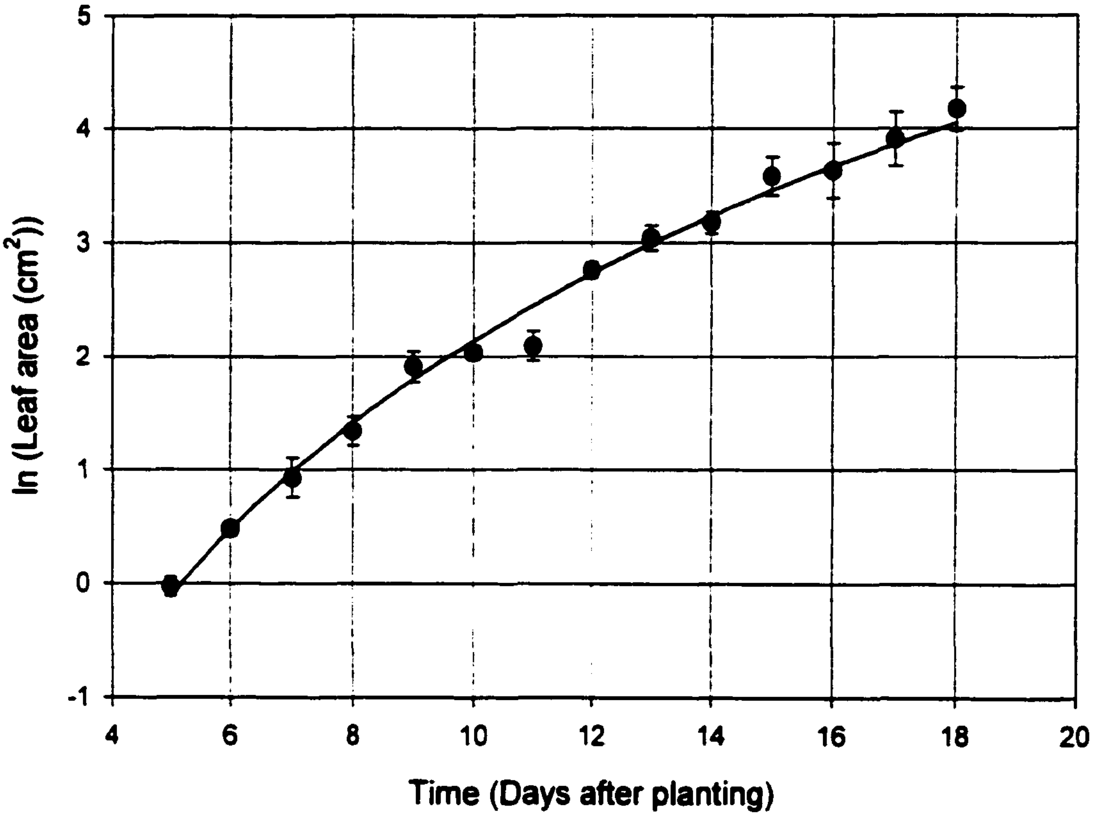

Figure 2.73. Leaf area of Colorado orange seeded proso millet biotype 5 to 18 days after planting. Points with bars represent means with standard error of the mean. Curve corresponds to the equation $Y = -5.3496 + 3.2516 * \ln(DAP)$ $r^2 = 0.94$.

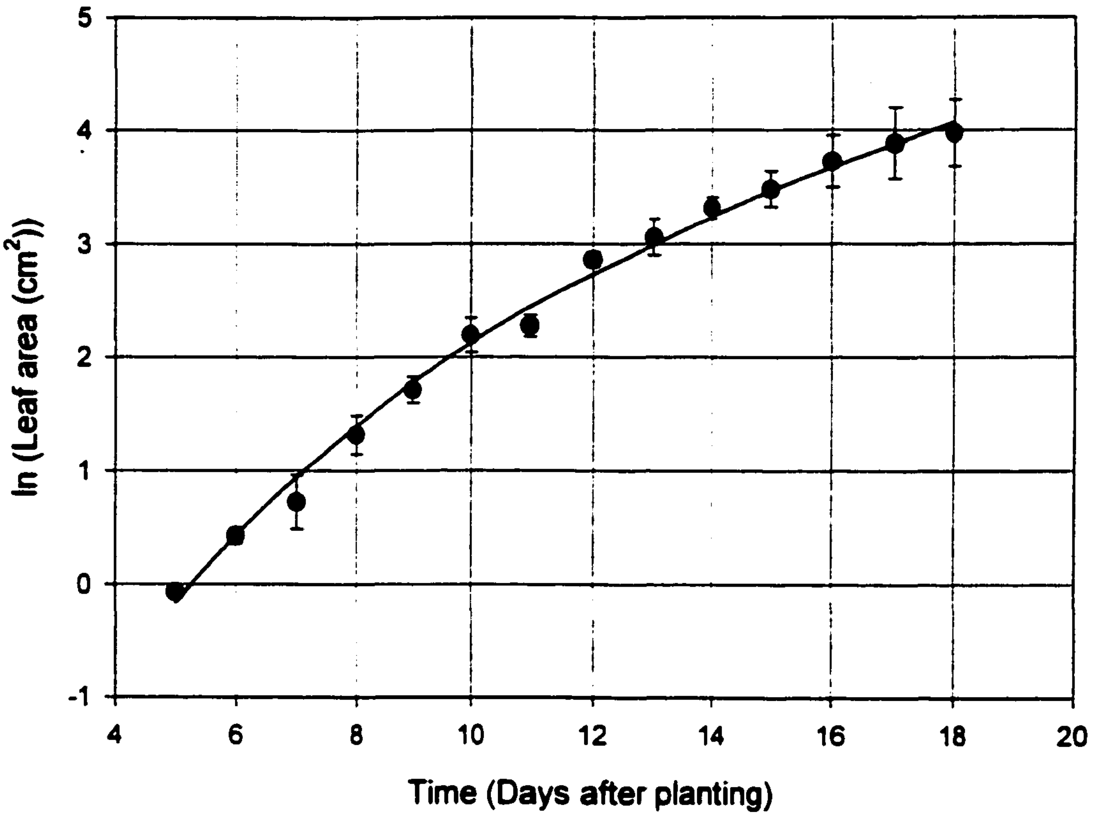

Figure 2.74. Leaf area of Colorado-Larimer white seeded proso millet biotype 5 to 18 days after planting. Points with bars represent means with standard error of the mean. Curve corresponds to the equation Y = -5.4898 + 3.3068*ln(DAP) r^2 = 0.93.

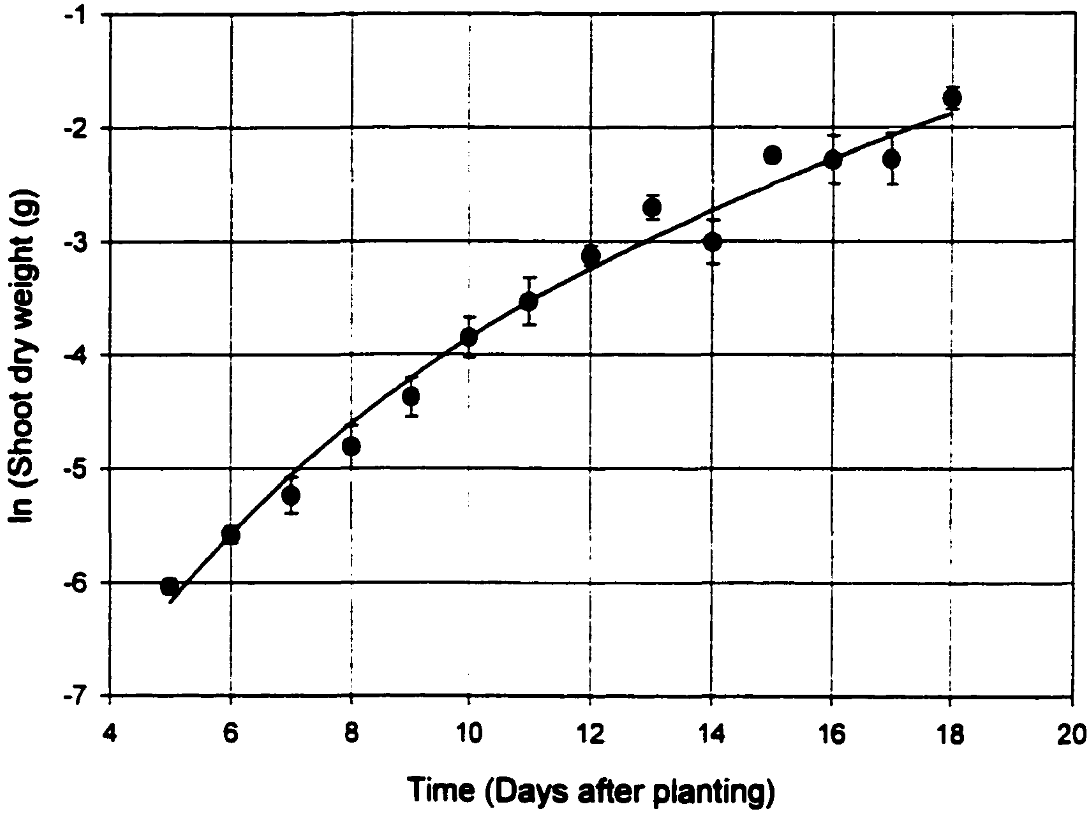

Figure 2.75. Shoot dry weight of Minnesota-Cambridge olive seeded proso millet biotype 5 to 18 days after planting. Points with vertical bars represent means with standard error of the mean. Curve corresponds to the equation Y = -11.5920 + 3.3605*ln(DAP) r^2 = 0.93.

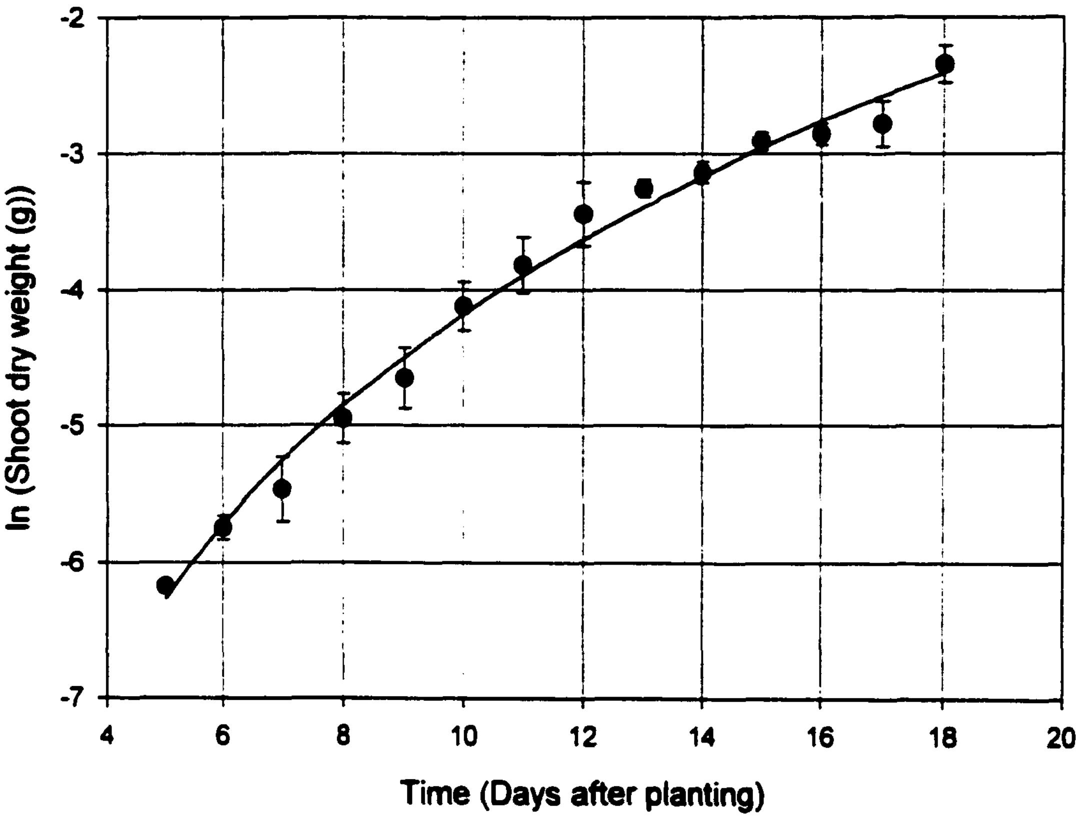

Figure 2.76. Shoot dry weight of Canada-Rosemount black seeded proso millet biotype 5 to 18 days after planting. Points with vertical bars represent means with standard error of the mean. Curve corresponds to the equation $Y = -11.1201 + 3.0141*\ln(DAP)$ $r^2 = 0.93$.

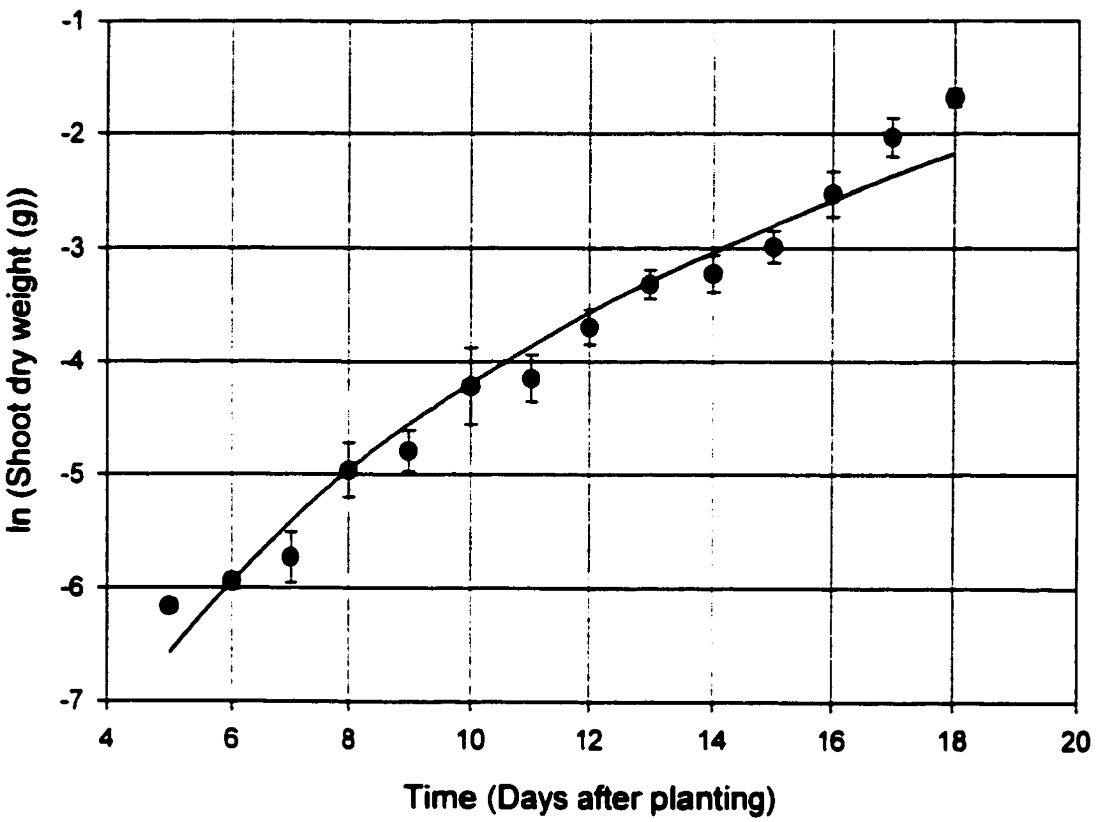

Figure 2.77. Shoot dry weight of Oregon-Grand Island olive seeded proso millet biotype 5 to 18 days after planting. Points with bars represent means with standard error of the mean. Curve corresponds to the equation Y = -12.0980 + 3.4324*ln(DAP) r^2 = 0.90.

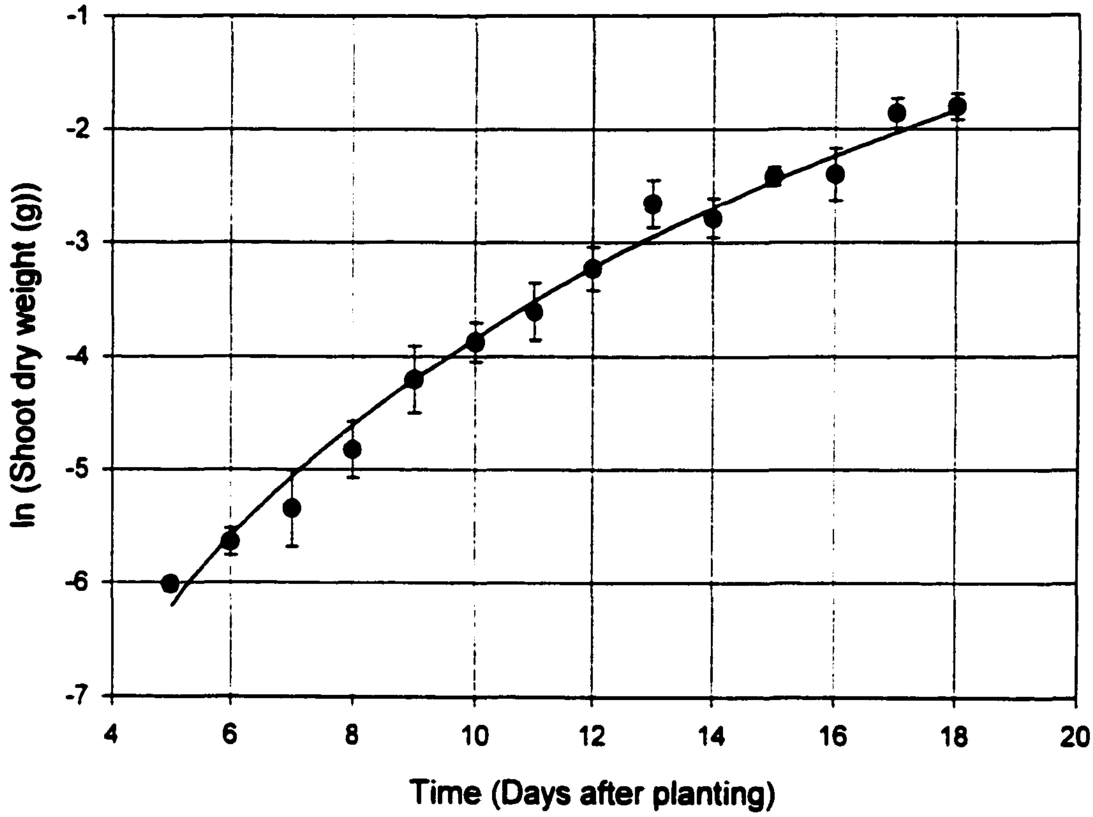

Figure 2.78. Leaf area of Ontario, Canada-Huron County black seeded proso millet biotype 5 to 18 days after planting. Points with bars represent means with the standard error of the mean. Curve corresponds to the equation $Y = -11.6986 + 3.4113*\ln(DAP)$ $r^2 = 0.91$.

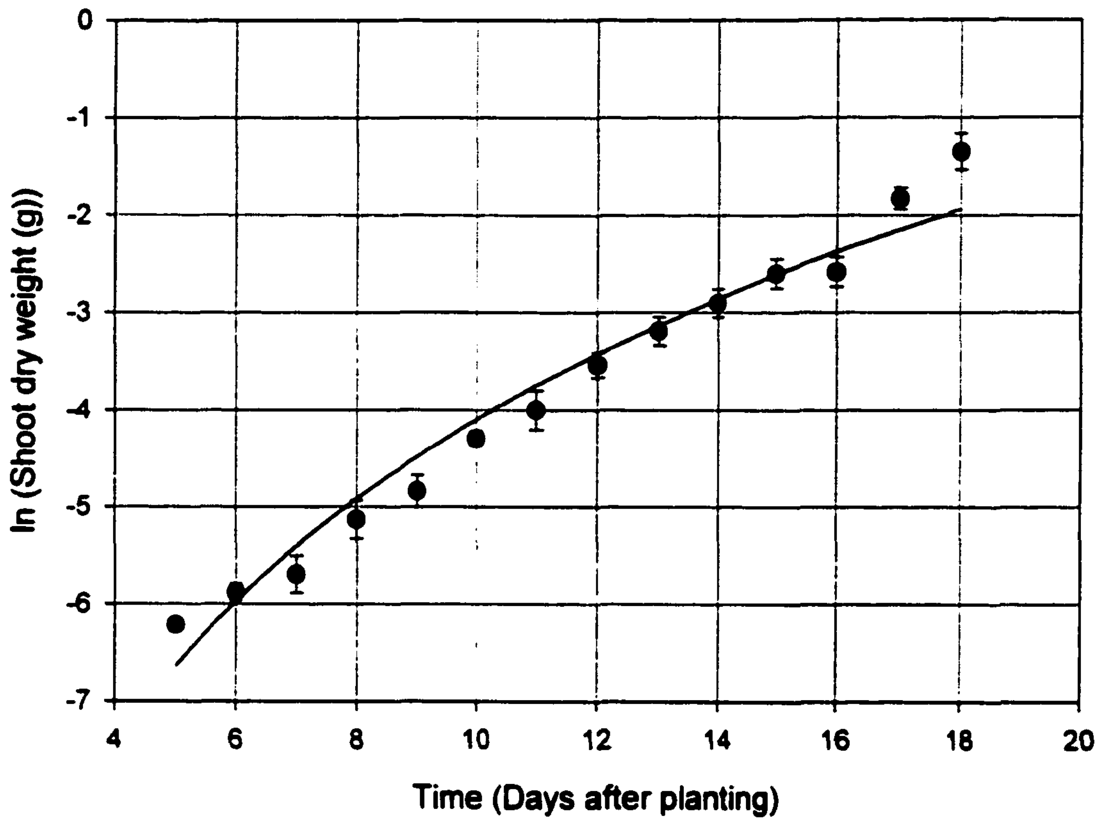

Figure 2.79. Shoot dry weight of Nebraska-Panhandle Center tan seeded proso millet biotype 5 to 18 days after planting. Points with bars represent means with standard error of the mean. Curve corresponds to the equation Y = -12.5407 + 3.6668*ln(DAP) r^2 = 0.93.

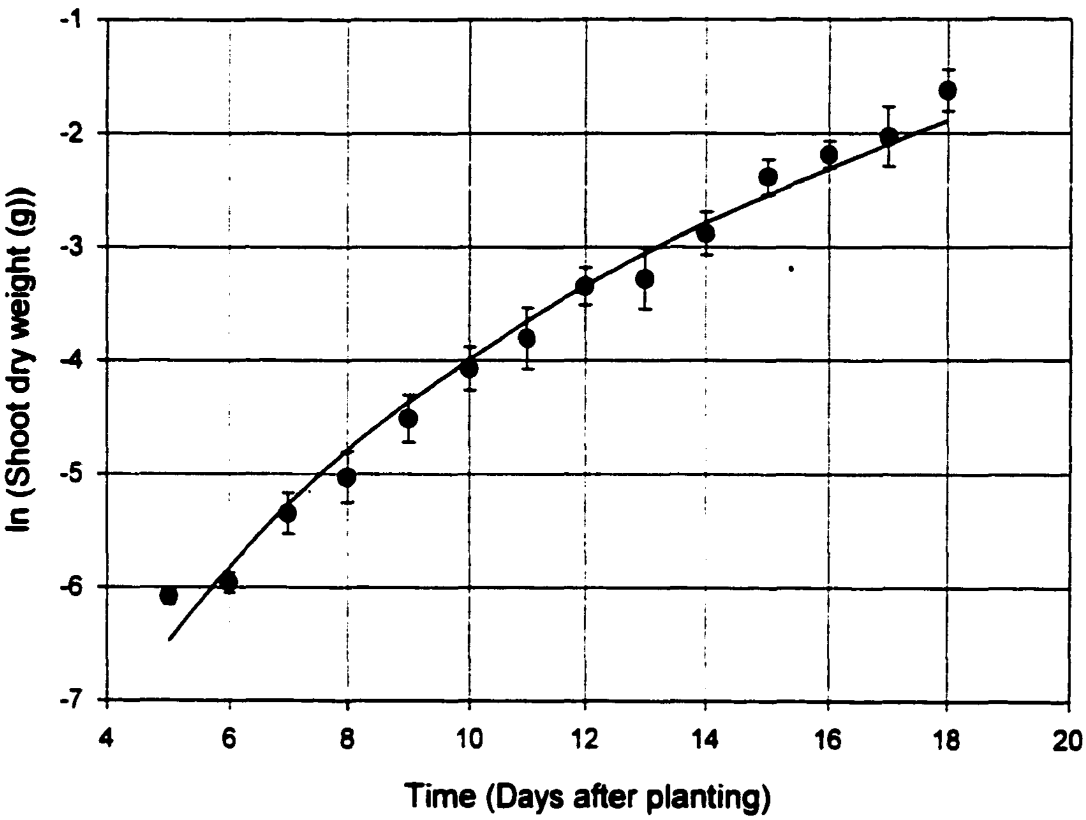

Figure 2.80. Shoot dry weight of Wyoming-Platte County brown seeded proso millet biotype 5 to 18 days after planting. Points with bars represent means with standard error of the mean. Curve corresponds to the equation $Y = -12.2334 + 3.5787*\ln(DAP)$ $r^2 = 0.91$.

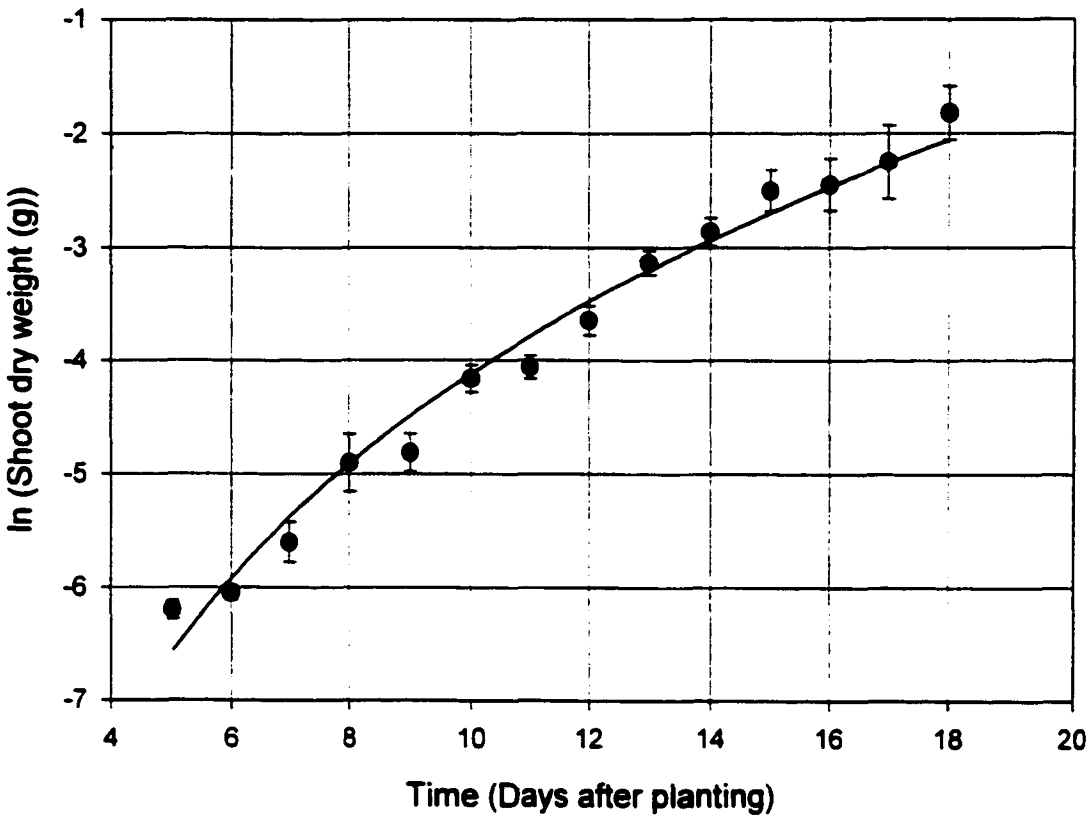

Figure 2.81. Shoot dry weight of Colorado-Weld County black seeded proso millet biotype 5 to 18 days after planting. Points with bars represent means with standard error of the mean. Curve corresponds to the equation $Y = -12.2160 + 3.5149*\ln(DAP)$ $r^2 = 0.92$.

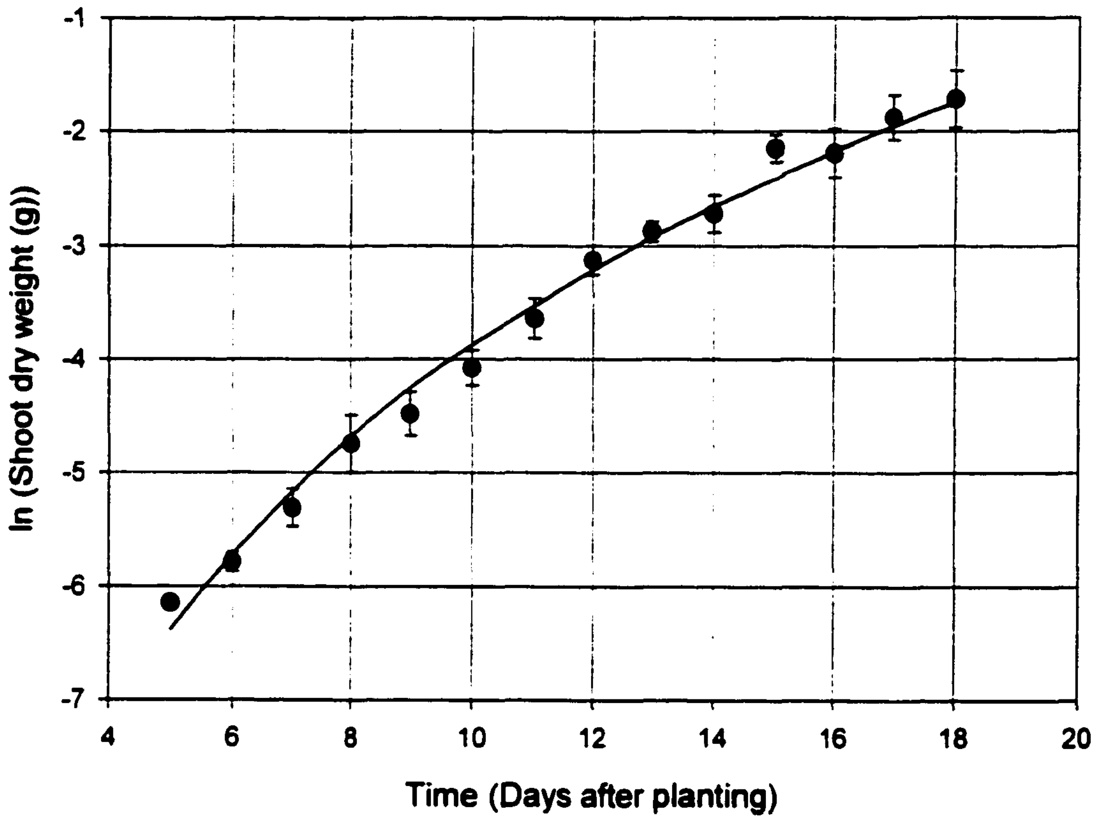

Figure 2.82. Shoot dry weight of Colorado-Weld County tan seeded proso millet biotype 5 to 18 days after planting. Points with bars represent means with standard error of the mean. Curve corresponds to the equation $Y = -12.1987 + 3.6161*\ln(DAP)$ $r^2 = 0.94$.

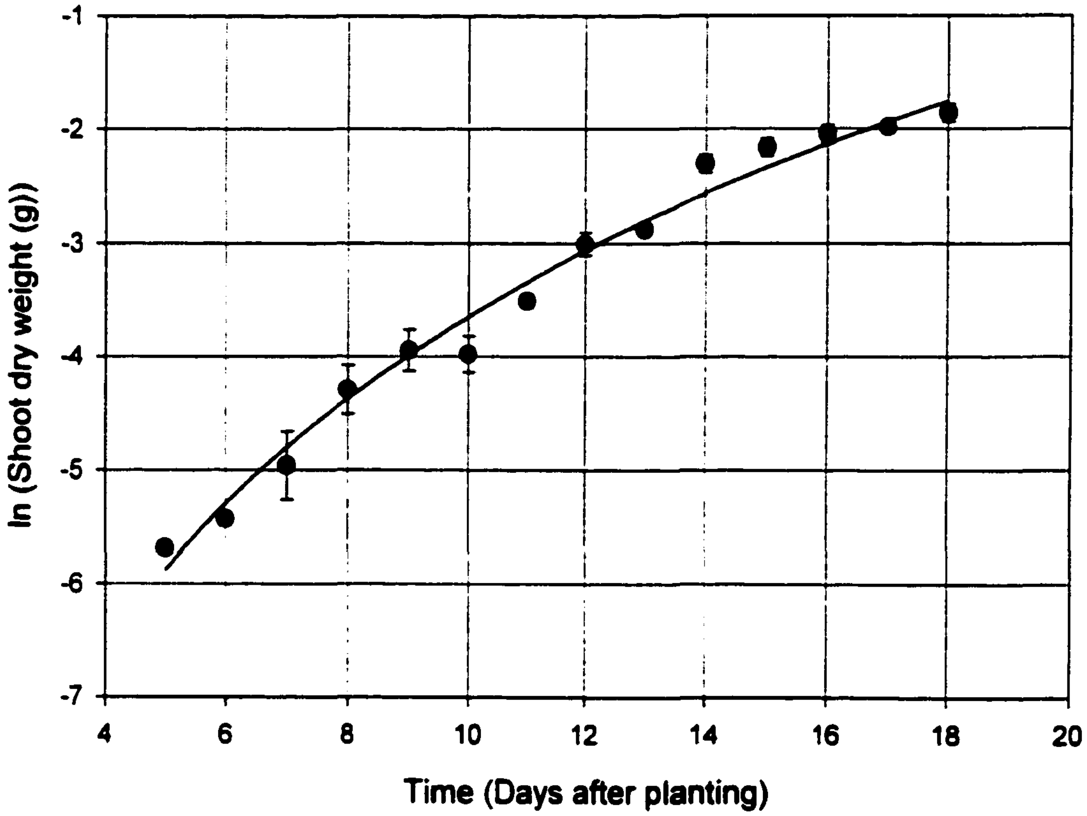

Figure 2.83. Shoot dry weight of South Dakota brown olive seeded proso millet biotype 5 to 18 days after planting. Points with bars represent means with standard error of the mean. Curve corresponds to the equation Y = -11.0709 + 3.2228*ln(DAP) r^2 = 0.94.

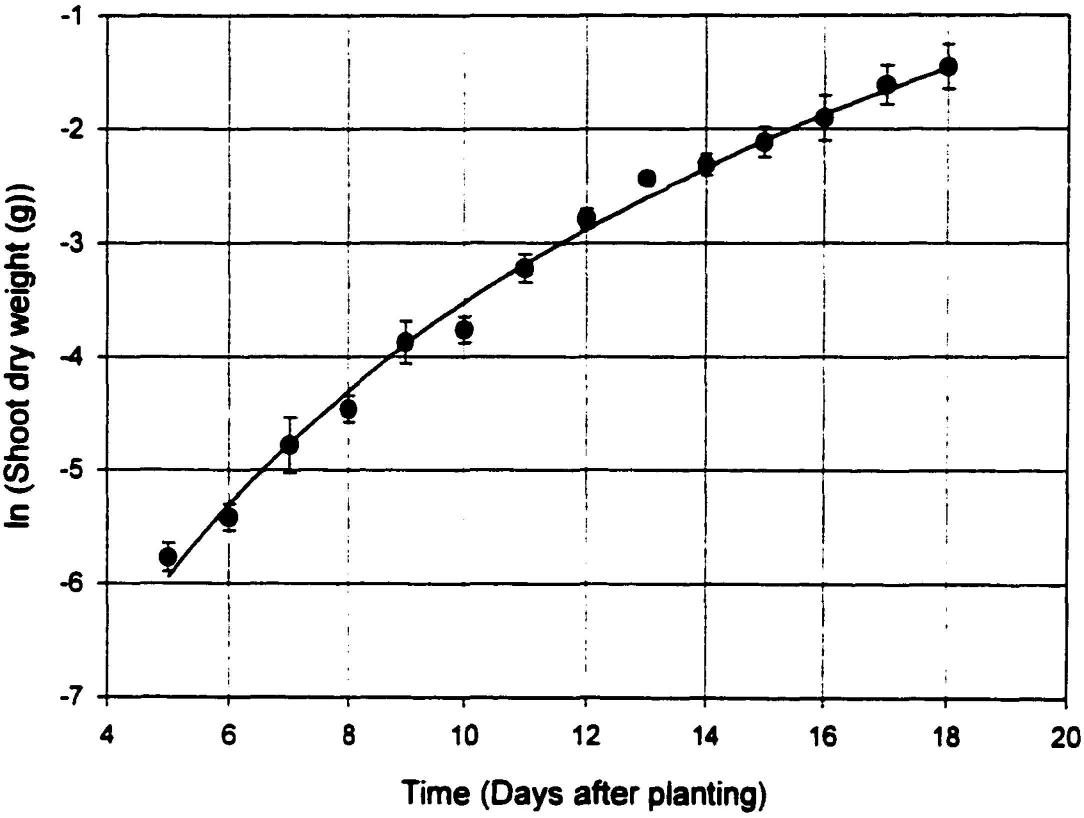

Figure 2.84. Shoot dry weight area of Colorado white seeded proso millet biotype 5 to 18 days after planting. Points with bars represent means with standard error of the mean. Curve corresponds to the equation Y = -11.5785 + 3.4996*ln(DAP) r^2 = 0.94.

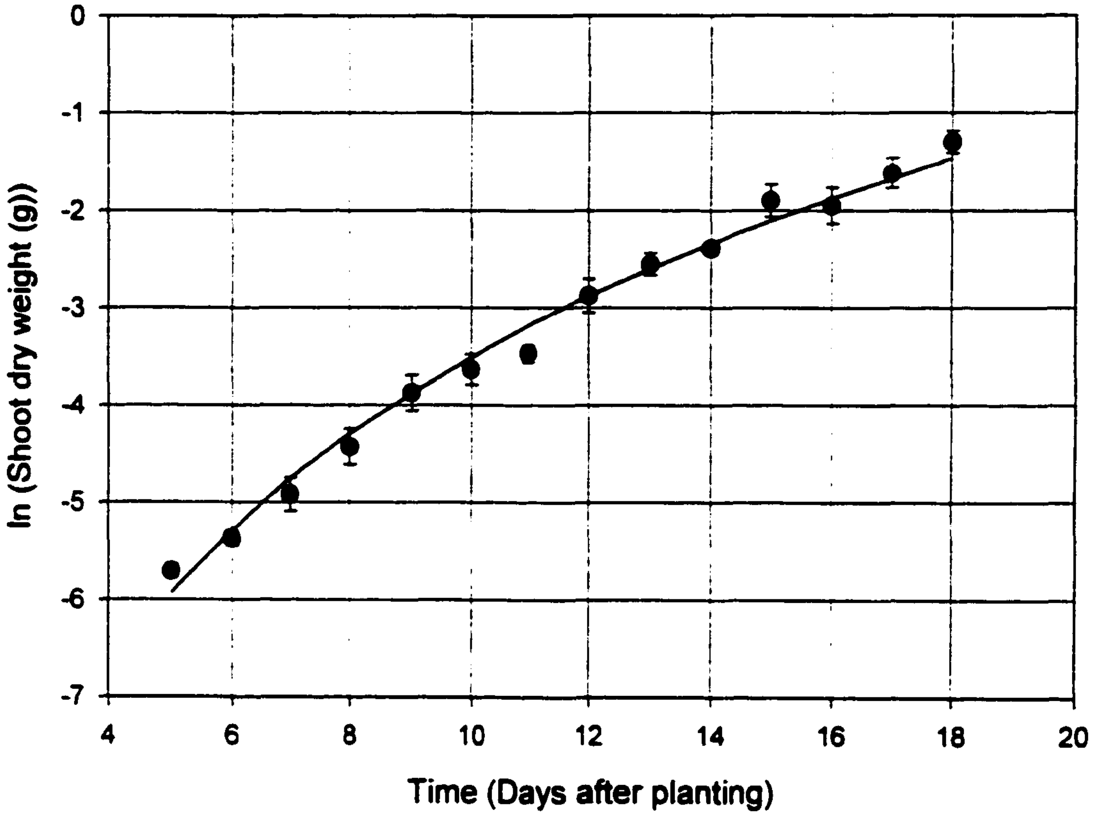

Figure 2.85. Shoot dry weight of Colorado orange seeded proso millet biotype 5 to 18 days after planting. Points with bars represent means with standard error of the mean. Curve corresponds to the equation Y = -11.5324 + 3.4813*ln(DAP) r^2 = 0.95.

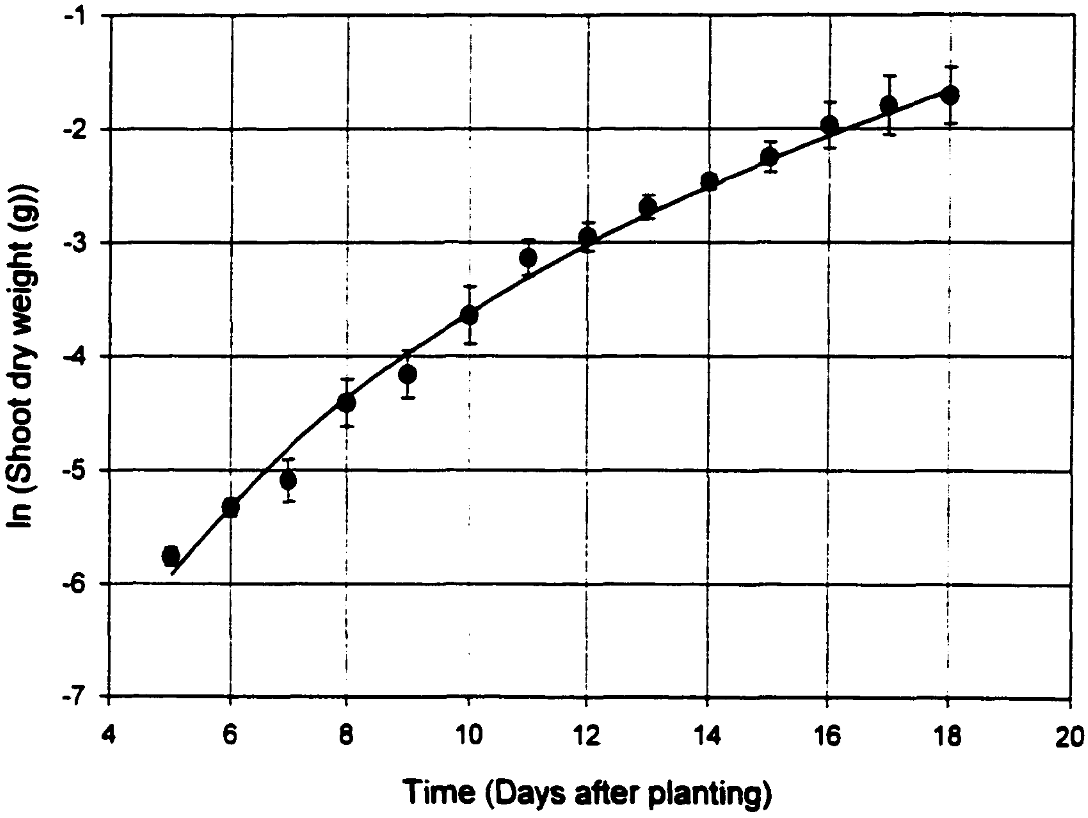

Figure 2.86. Shoot dry weight of Colorado-Larimer white seeded proso millet biotype 5 to 18 days after planting. Points with bars represent means with standard error of the mean. Curve corresponds to the equation $Y = -11.2779 + 3.3237*\ln(DAP)$ $r^2 = 0.92$.

APPENDICES

Appendix 2.1.

Differences detected by the confidence intervals estimated from the Richards function for shoot dry weight. Numbers in the table are the number of biotype that had a statiscally higher value. B indicates differences detected in some harvests, but not throughout the experiment. Biotype number is the number of accession presented in Table 2.1.

HARVEST	BIOTYPE	SHOOT DRY WEIGHT											
		BIOTYPE											
		1	4	8	9	10	12	13	15	16	17	18	21
GENERAL	1		1	-	-	-	-	B	-	-	-	B	-
	4	1		B	B	B	-	-	B	-	-	-	B
	8	-	B		-	-	B	B	-	-	B	B	-
	9	-	B	-		-	B	B	-	-	-	B	-
	10	-	B	-	-		-	B	-	-	-	-	-
	12	-	-	B	B	-		-	B	-	-	-	-
	13	B	-	B	B	B	-		B	B	-	-	B
	15	-	B	-	-	-	B	B		-	-	B	-
	16	-	-	-	-	-	-	B	-		-	-	-
	17	-	-	B	-	-	-	-	-	-		-	-
	18	B	-	B	B	-	-	-	B	-	-		-
	21	-	B	-	-	-	-	B	-	-	-	-	
		1	4	8	9	10	12	13	15	16	17	18	21
14	1		1	-	-	-	-	1	-	-	-	-	-
	4	1		-	-	-	-	-	-	-	-	-	-
	8	-	-		-	-	-	-	-	-	-	-	-
	9	-	-	-		-	9	9	-	-	-	9	-
	10	-	-	-	-		-	-	-	-	-	-	-
	12	-	-	-	9	-		-	-	-	-	-	-
	13	1	-	-	9	-	-		-	16	-	-	-
	15	-	-	-	-	-	-	-		-	-	-	-
	16	-	-	-	-	-	-	16	-		-	-	-
	17	-	-	-	-	-	-	-	-	-		-	-
	18	-	-	-	9	-	-	-	-	-	-		-
	21	-	-	-	-	-	-	-	-	-	-	-	

		1	4	8	9	10	12	13	15	16	17	18	21
26	1		1	-	-	-	-	-	-	-	-	-	-
	4	1		-	-	-	-	-	-	-	-	-	-
	8	-	-		-	-	-	-	-	-	-	-	-
	9	-	-	-		-	-	-	-	-	-	-	-
	10	-	-	-	-		-	-	-	-	-	-	-
	12	-	-	-	-	-		-	-	-	-	-	-
	13	-	-	-	-	-	-		-	-	-	-	-
	15	-	-	-	-	-	-	-		-	-	-	-
	16	-	-	-	-	-	-	-	-		-	-	-
	17	-	-	-	-	-	-	-	-	-		-	-
	18	-	-	-	-	-	-	-	-	-	-		-
	21	-	-	-	-	-	-	-	-	-	-	-	
		1	4	8	9	10	12	13	15	16	17	18	21
42	1		1	-	-	-	-	-	-	-	-	-	-
	4	1		-	-	-	-	-	-	-	-	-	-
	8	-	-		-	-	-	-	-	-	-	-	-
	9	-	-	-		-	-	-	-	-	-	-	-
	10	-	-	-	-		-	-	-	-	-	-	-
	12	-	-	-	-	-		-	-	-	-	-	-
	13	-	-	-	-	-	-		-	-	-	-	-
	15	-	-	-	-	-	-	-		-	-	-	-
	16	-	-	-	-	-	-	-	-		-	-	-
	17	-	-	-	-	-	-	-	-	-		-	-
	18	-	-	-	-	-	-	-	-	-	-		-
	21	-	-	-	-	-	-	-	-	-	-	-	
		1	4	8	9	10	12	13	15	16	17	18	21
56	1		1	-	-	-	-	1	-	-	-	1	-
	4	1		8	9	10	-	-	15	-	-	-	21
	8	-	8		-	-	8	8	-	-	8	8	-
	9	-	9	-		-	-	-	-	-	-	-	-
	10	-	10	-	-		-	10	-	-	-	-	-
	12	-	-	8	-	-		-	15	-	-	-	-
	13	1	-	8	-	10	-		15	16	-	-	21
	15	-	15	-	-	-	15	15		-	-	15	-
	16	-	-	-	-	-	-	16	-		-	-	-
	17	-	-	8	-	-	-	-	-	-		-	-
	18	1	-	8	-	-	-	-	15	-	-		-
	21	-	21	-	-	-	-	21	-	-	-	-	

		1	4	8	9	10	12	13	15	16	17	18	21
	1		1	-	-	-	-	-	-	-	-	-	-
	4	1		8	9	10	-	-	15	-	-	-	21
	8	-	8		-	-	-	8	-	-	-	-	-
	9	-	9	-		-	-	-	-	-	-	-	-
	10	-	10	-	-		-	-	-	-	-	-	-
70	12	-	-	-	-	-		-	-	-	-	-	-
	13	-	-	8	-	-	-		15	-	-	-	-
	15	-	15	-	-	-	-	15		-	-	-	-
	16	-	-	-	-	-	-	-	-		-	-	-
	17	-	-	-	-	-	-	-	-	-		-	-
	18	-	-	-	-	-	-	-	-	-	-		-
	21	-	21	-	-	-	-	-	-	-	-	-	

Appendix 2.2.

Differences detected by the confidence intervals estimated from the Richards function for leaf area. Numbers in the table are the number of biotype that had a statiscally higher value. B indicates differences detected in some harvests, but not throughout the experiment. Biotype number is the number of accession presented in Table 2.1.

HARVEST	BIOTYPE	LEAF AREA											
		BIOTYPE											
		1	4	8	9	10	12	13	15	16	17	18	21
GENERAL	1		B	-	-	-	-	B	-	B	-	B	-
	4	B		B	B	B	-	-	B	-	B	-	-
	8	-	B		-	-	-	B	-	B	-	B	-
	9	-	B	-		-	-	B	-	-	-	-	-
	10	-	B	-	-		B	B	-	B	B	B	-
	12	-	-	-	-	B		-	B	-	-	-	-
	13	B	-	B	B	B	-		B	-	B	-	-
	15	-	B	-	-	-	B	B		B	-	B	-
	16	B	-	B	-	B	-	-	B		-	-	-
	17	-	B	-	-	B	-	B	-	-		-	-
	18	B	-	B	-	B	-	-	B	-	-		-
	21	-	-	-	-	-	-	-	-	-	-	-	
		1	4	8	9	10	12	13	15	16	17	18	21
14	1		-	-	-	-	-	-	-	-	-	-	-
	4	-		-	-	-	-	-	-	-	-	-	-
	8	-	-		-	-	-	-	-	-	-	-	-
	9	-	-	-		-	-	-	-	-	-	-	-
	10	-	-	-	-		-	-	-	-	-	-	-
	12	-	-	-	-	-		-	-	-	-	-	-
	13	-	-	-	-	-	-		-	-	-	-	-
	15	-	-	-	-	-	-	-		-	-	-	-
	16	-	-	-	-	-	-	-	-		-	-	-
	17	-	-	-	-	-	-	-	-	-		-	-
	18	-	-	-	-	-	-	-	-	-	-		-
	21	-	-	-	-	-	-	-	-	-	-	-	

		1	4	8	9	10	12	13	15	16	17	18	21
26	1		-	-	-	-	-	-	-	-	-	-	-
	4	-		-	-	-	-	-	-	-	-	-	-
	8	-	-		-	-	-	-	-	-	-	-	-
	9	-	-	-		-	-	-	-	-	-	-	-
	10	-	-	-	-		-	-	-	-	-	-	-
	12	-	-	-	-	-		-	-	-	-	-	-
	13	-	-	-	-	-	-		-	-	-	-	-
	15	-	-	-	-	-	-	-		-	-	-	-
	16	-	-	-	-	-	-	-	-		-	-	-
	17	-	-	-	-	-	-	-	-	-		-	-
	18	-	-	-	-	-	-	-	-	-	-		-
	21	-	-	-	-	-	-	-	-	-	-	-	
		1	4	8	9	10	12	13	15	16	17	18	21
42	1		1	-	-	-	-	1	-	-	-	-	-
	4	1		8	-	10	-	-	15	-	-	-	-
	8	-	8		-	-	-	8	-	-	-	8	-
	9	-	-	-		-	-	-	-	-	-	-	-
	10	-	10	-	-		-	-	-	-	-	10	-
	12	-	-	-	-	-		-	-	-	-	-	-
	13	1	-	8	-	10	-		15	-	-	-	-
	15	-	15	-	-	-	-	15		-	-	15	-
	16	-	-	-	-	-	-	-	-		-	-	-
	17	-	-	-	-	-	-	-	-	-		-	-
	18	-	-	8	-	10	-	-	15	-	-		-
	21	-	-	-	-	-	-	-	-	-	-	-	
		1	4	8	9	10	12	13	15	16	17	18	21
56	1		1	-	-	-	-	1	-	1	-	1	-
	4	1		8	9	10	-	-	15	-	17	-	21
	8	-	8		-	-	-	8	-	8	-	8	-
	9	-	9	-		-	-	13	-	-	-	-	-
	10	-	10	-	-		-	10	-	10	-	10	-
	12	-	-	-	-	-		-	15	-	-	-	-
	13	1	-	8	13	10	-		15	-	17	-	-
	15	-	15	-	-	-	15	15		15	-	15	-
	16	1	-	8	-	10	-	-	15		-	-	-
	17	-	17	-	-	-	-	17	-	-		-	-
	18	1	-	8	-	10	-	-	15	-	-		-
	21	-	21	-	-	-	-	-	-	-	-	-	

		1	4	8	9	10	12	13	15	16	17	18	21
	1		1	-	-	-	-	1	-	-	-	1	-
	4	1		8	9	10	-	-	15	-	17	-	-
	8	-	8		-	-	-	8	-	8	-	8	-
	9	-	9	-		-	-	-	-	-	-	-	-
	10	-	10	-	-		10	10	-	-	10	10	-
70	12	-	-	-	-	10		-	-	-	-	-	-
	13	1	-	8	-	10	-		15	-	-	-	-
	15	-	15	-	-	-	-	15		15	-	15	-
	16	-	-	8	-	-	-	-	15		-	-	-
	17	-	17	-	-	10	-	-	-	-		-	-
	18	1	-	8	-	10	-	-	15	-	-		-
	21	-	-	-	-	-	-	-	-	-	-	-	

Appendix 2.3.

Differences detected by the confidence intervals estimated from the Richards function for plant height. Numbers in the table are the number of biotype that had a statiscally higher value. B indicates differences detected in some harvests, but not throughout the experiment. Biotype number is the number of accession presented in Table 2.1.

HARVEST	BIOTYPE	HEIGHT											
		BIOTYPE											
		1	4	8	9	10	12	13	15	16	17	18	21
GENERAL	1		-	-	-	-	-	-	-	-	-	B	B
	4	-		B	-	-	-	-	-	-	-	B	B
	8	-	B		-	-	-	B	-	B	B	B	-
	9	-	-	-		-	-	-	-	-	B	B	-
	10	-	-	-	-		-	-	-	-	-	B	B
	12	-	-	-	-	-		-	-	-	-	-	B
	13	-	-	B	-	-	-		-	-	-	-	B
	15	-	-	-	-	-	-	-		-	-	B	-
	16	-	-	B	-	-	-	-	-		-	B	B
	17	-	-	B	B	-	-	-	-	-		-	B
	18	B	B	B	B	B	-	-	B	B	-		B
	21	B	B	-	-	B	B	B	-	B	B	B	
		1	4	8	9	10	12	13	15	16	17	18	21
14	1		-	-	-	-	-	-	-	-	-	-	-
	4	-		-	-	-	-	-	-	-	-	-	-
	8	-	-		-	-	-	-	-	-	-	-	-
	9	-	-	-		-	-	-	-	-	-	-	-
	10	-	-	-	-		-	-	-	-	-	-	-
	12	-	-	-	-	-		-	-	-	-	-	-
	13	-	-	-	-	-	-		-	-	-	-	-
	15	-	-	-	-	-	-	-		-	-	-	-
	16	-	-	-	-	-	-	-	-		-	-	-
	17	-	-	-	-	-	-	-	-	-		-	-
	18	-	-	-	-	-	-	-	-	-	-		-
	21	-	-	-	-	-	-	-	-	-	-	-	

		1	4	8	9	10	12	13	15	16	17	18	21
26	1		-	-	-	-	-	-	-	-	-	-	-
	4	-		-	-	-	-	-	-	-	-	-	21
	8	-	-		-	-	-	-	-	-	-	-	-
	9	-	-	-		-	-	-	-	-	-	-	-
	10	-	-	-	-		-	-	-	-	-	-	-
	12	-	-	-	-	-		-	-	-	-	-	21
	13	-	-	-	-	-	-		-	-	-	-	-
	15	-	-	-	-	-	-	-		-	-	-	-
	16	-	-	-	-	-	-	-	-		-	-	-
	17	-	-	-	-	-	-	-	-	-		-	21
	18	-	-	-	-	-	-	-	-	-	-		-
	21	-	21	-	-	-	-	21	-	-	21	-	

		1	4	8	9	10	12	13	15	16	17	18	21
42	1		-	-	-	-	-	-	-	-	-	1	-
	4	-		8	-	-	-	-	-	-	-	4	21
	8	-	8		-	-	-	8	-	-	8	8	-
	9	-	-	-		-	-	-	-	-	9	9	-
	10	-	-	-	-		-	-	-	-	-	10	-
	12	-	-	-	-	-		-	-	-	-	-	21
	13	-	-	8	-	-	-		-	-	-	-	21
	15	-	-	-	-	-	-	-		-	-	15	-
	16	-	-	-	-	-	-	-	-		-	16	21
	17	-	-	8	9	-	-	-	-	-		-	21
	18	1	4	8	9	10	-	-	15	16	-		21
	21	-	21	-	-	-	21	21	-	21	21	21	

		1	4	8	9	10	12	13	15	16	17	18	21
56	1		-	-	-	-	-	-	-	-	-	1	-
	4	-		8	-	-	-	-	-	-	-	4	21
	8	-	8		-	-	-	8	-	8	8	8	-
	9	-	-	-		-	-	-	-	-	9	9	-
	10	-	-	-	-		-	-	-	-	-	10	21
	12	-	-	-	-	-		-	-	-	-	-	21
	13	-	-	8	-	-	-		-	-	-	-	21
	15	-	-	-	-	-	-	-		-	-	15	-
	16	-	-	8	-	-	-	-	-		-	16	21
	17	-	-	8	9	-	-	-	-	-		-	21
	18	1	4	8	9	10	-	-	15	16	-		21
	21	-	21	-	-	21	21	21	-	21	21	21	

		1	4	8	9	10	12	13	15	16	17	18	21
	1		-	-	-	-	-	-	-	-	-	1	21
	4	-		8	-	-	-	-	-	-	-	4	21
	8	-	8		-	-	-	8	-	8	8	8	-
	9	-	-	-		-	-	-	-	-	9	9	-
	10	-	-	-	-		-	-	-	-	-	10	21
70	12	-	-	-	-	-		-	-	-	-	-	21
	13	-	-	8	-	-	-		-	-	-	-	21
	15	-	-	-	-	-	-	-		-	-	15	-
	16	-	-	8	-	-	-	-	-		-	18	21
	17	-	-	8	9	-	-	-	-	-		-	21
	18	1	4	8	9	10	-	-	15	18	-		21
	21	21	21	-	-	21	21	21	-	21	21	21	

Appendix 2.4.

Differences detected by the confidence intervals estimated from the logarithmic function for shoot dry weight. Number in the table are the number of biotypes that had a statistically greater value. B indicates differences detected in some harvests, but not throughout the experiment. Biotype number is the accession number presented in Table 2.1.

HARVEST	BIOTIPE	SHOOT DRY WEIGHT											
		BIOTYPE											
		1	4	8	9	10	12	13	15	16	17	18	21
	1		B	B	-	B	-	B	-	B	B	B	B
	4	B		-	B	B	B	B	B	16	17	18	21
	8	B	-		B	-	B	-	B	16	17	18	21
	9	-	B	B		B	-	B	-	B	B	B	B
	10	B	B	-	B		-	-	B	B	17	18	B
GENERAL	12	-	B	B	-	-		-	-	B	17	18	B
	13	B	B	-	B	-	-		B	16	17	18	21
	15	-	B	B	-	B	-	B		B	17	18	21
	16	B	16	16	B	B	B	16	B		B	B	-
	17	B	17	17	B	17	17	17	17	B		-	-
	18	B	18	18	B	18	18	18	18	B	-		-
	21	B	21	21	B	B	B	21	21	-	-	-	
		1	4	8	9	10	12	13	15	16	17	18	21
	1		-	1	-	1	-	1	-	16	-	-	-
	4	-		-	-	-	-	-	-	16	17	18	21
	8	1	-		-	-	-	-	-	16	17	18	21
	9	-	-	-		-	-	9	-	16	-	-	-
	10	1	-	-	-		-	-	-	16	17	18	21
5	12	-	-	-	-	-		-	-	16	17	18	21
	13	1	-	-	9	-	-		-	16	17	18	21
	15	-	-	-	-	-	-	-		16	17	18	21
	16	16	16	16	16	16	16	16	16		-	-	-
	17	-	17	17	-	17	17	17	17	-		-	-
	18	-	18	18	-	18	18	18	18	-	-		-
	21	-	21	21	-	21	21	21	21	-	-	-	

		1	4	8	9	10	12	13	15	16	17	18	21
6	1		-	1	-	1	-	1	-	16	17	18	21
	4	-		-	-	-	-	-	-	16	17	18	21
	8	1	-		9	-	-	-	-	16	17	18	21
	9	-	-	9		9	-	9	-	16	17	18	-
	10	1	-	-	9		-	-	-	16	17	18	21
	12	-	-	-	-	-		-	-	16	17	18	21
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	15	-	-	-	-	-	-	-		16	17	18	21
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		1	4	8	9	10	12	13	15	16	17	18	21
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	8	1	-		9	-	-	-	15	16	17	18	21
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	13	-	13	-	-	-	-		15	16	17	18	21
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	16	-	16	16	-	-	-	16	-		17	18	-
	17	17	17	17	17	17	17	17	17	17		-	-
	18	18	18	18	18	18	18	18	18	18	-		-
	21	21	21	21	-	-	21	21	-	-	-	-	

Appendix 2.5.

Differences detected by the confidence intervals estimated from the logarithmic function for leaf area. Number in the table are the number of biotypes that had a statistically greater value. B indicates differences detected in some harvests, but not throughout the experiment. Biotype number is the accession number presented in Table 2.1.

HARVEST	BIOTYPE	LEAF AREA											
		BIOTYPE											
		1	4	8	9	10	12	13	15	16	17	18	21
GENERAL	1		B	B	-	B	-	B	-	B	B	B	B
	4	B		-	B	B	B	B	B	16	17	18	21
	8	B	B		B	-	B	-	B	16	17	18	21
	9	-	B	8		B	-	B	-	B	B	B	B
	10	B	B	B	B		-	B	B	B	17	18	21
	12	B	B	B	B	B		-	-	B	17	18	B
	13	-	B	B	-	B	-		B	16	17	18	21
	15	-	B	B	-	B	-	-		B	17	18	B
	16	B	16	16	B	B	B	B	B		B	B	-
	17	17	17	17	17	17	17	17	17	B		-	-
	18	18	18	18	18	18	18	18	B	B	-		-
	21	21	21	21	21	21	21	21	B	B	-	-	
		1	4	8	9	10	12	13	15	16	17	18	21
5	1		-	1	-	1	1	-	-	-	17	18	21
	4	-		4	-	4	4	-	-	16	17	18	21
	8	1	4		8	-	-	-	-	16	17	18	21
	9	-	-	8		9	9	-	-	16	17	18	21
	10	1	4	-	9		12	13	15	16	17	18	21
	12	1	4	-	9	12		-	-	16	17	18	21
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	15	-	-	-	-	15	-	-		16	17	18	21
	16	-	16	16	16	16	16	16	16		-	-	-
	17	17	17	17	17	17	17	17	17	-		-	-
	18	18	18	18	18	18	18	18	18	-	-		-
	21	21	21	21	21	21	21	21	21	-	-	-	

		1	4	8	9	10	12	13	15	16	17	18	21
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	4	-		4	-	4	4	-	-	16	17	18	21
	8	1	4		8	-	-	-	-	16	17	18	21
	9	-	-	8		9	9	-	-	16	17	18	21
	10	1	4	-	9		12	13	15	16	17	18	21
6	12	1	4	-	9	12		-	-	16	17	18	21
	13	-	-	-	-	13	-		-	16	17	18	21
	15	-	-	-	-	15	-	-		16	17	18	21
	16	16	16	16	16	16	16	16	16		-	-	-
	17	17	17	17	17	17	17	17	17	-		-	-
	18	18	18	18	18	18	18	18	18	-	-		-
	21	21	21	21	21	21	21	21	21	-	-	-	

		1	4	8	9	10	12	13	15	16	17	18	21
	1		-	1	-	1	1	-	-	16	17	18	21
	4	-		4	-	4	-	-	-	16	17	18	21
	8	1	4		8	-	-	-	-	16	17	18	21
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	17	17	17	17	17	17	17	17	17	-		-	-
	18	18	18	18	18	18	18	18	18	-	-		-
	21	21	21	21	21	21	21	21	21	-	-	-	

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	4	-		4	-	4	-	-	-	16	17	18	21
	8	1	4		8	-	-	-	15	16	17	18	21
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	17	17	17	17	17	17	17	17	17	-		-	-
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	4	-		4	9	4	-	-	-	16	17	18	21
	8	1	4		8	-	-	-	15	16	17	18	21
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	21	21	21	21	21	21	21	21	21	-	-	-	

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	4	1		-	9	-	-	-	-	16	17	18	21
	8	1	-		8	-	-	13	15	16	17	18	21
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	16	16	16	16	16	16	16	16	16		17	18	21
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	21	21	21	21	21	21	21	21	21	21	-	-	

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	4	1		-	9	-	-	-	15	16	17	18	21
	8	1	-		8	-	8	13	15	16	17	18	21
	9	-	9	8		9	-	-	-	-	17	18	21
	10	1	-	-	9		-	-	15	16	17	18	21
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	16	16	16	16	-	16	16	16	16		17	18	21
	17	17	17	17	17	17	17	17	17	17		-	-
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	21	21	21	21	21	21	21	21	21	21	-	-	

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	8	1	-		8	-	8	13	15	16	17	18	21
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	4	1		-	9	10	12	13	15	16	17	18	21
	8	1	-		8	10	8	13	15	16	17	18	21
	9	-	9	8		-	-	-	-	-	17	18	21
	10	-	10	10	-		-	-	-	16	17	18	21
14	12	-	12	8	-	-		-	-	-	17	18	21
	13	-	13	13	-	-	-		-	-	17	18	21
	15	-	15	15	-	-	-	-		-	17	18	21
	16	16	16	16	-	16	-	-	-		17	18	21
	17	17	17	17	17	17	17	17	17	17		-	-
	18	18	18	18	18	18	18	18	18	18	-		-
	21	21	21	21	21	21	21	21	21	21	-	-	

15		1	4	8	9	10	12	13	15	16	17	18	21
	1		1	1	-	-	-	-	-	16	17	18	21
	4	1		-	9	10	12	13	15	16	17	18	21
	8	1	-		8	10	8	13	15	16	17	18	21
	9	-	9	8		-	-	-	-	-	17	18	21
	10	-	10	10	-		-	-	-	16	17	18	21
	12	-	12	8	-	-		-	-	-	17	18	21
	13	-	13	13	-	-	-		-	-	17	18	21
	15	-	15	15	-	-	-	-		-	17	18	21
	16	16	16	16	-	16	-	-	-		17	18	21
	17	17	17	17	17	17	17	17	17	17		-	-
	18	18	18	18	18	18	18	18	18	18	-		-
	21	21	21	21	21	21	21	21	21	21	-	-	

16		1	4	8	9	10	12	13	15	16	17	18	21
	1		1	-	-	-	-	-	-	-	17	18	21
	4	1		-	9	10	12	13	15	16	17	18	21
	8	-	-		8	10	8	13	15	16	17	18	21
	9	-	9	8		-	-	-	-	-	17	18	21
	10	-	10	10	-		-	-	-	-	17	18	21
	12	-	12	8	-	-		-	-	-	17	18	21
	13	-	13	13	-	-	-		-	-	17	18	21
	15	-	15	15	-	-	-	-		-	17	18	21
	16	-	16	16	-	-	-	-	-		17	18	21
	17	17	17	17	17	17	17	17	17	17		-	-
	18	18	18	18	18	18	18	18	18	18	-		-
	21	21	21	21	21	21	21	21	21	21	-	-	

17		1	4	8	9	10	12	13	15	16	17	18	21
	1		1	-	-	-	-	-	-	-	17	18	21
	4	1		-	9	10	12	13	15	16	17	18	21
	8	-	-		8	10	8	13	15	16	17	18	21
	9	-	9	8		-	-	-	-	-	17	18	21
	10	-	10	10	-		-	-	-	-	17	18	21
	12	-	12	8	-	-		-	-	-	17	18	21
	13	-	13	13	-	-	-		-	-	17	18	21
	15	-	15	15	-	-	-	-		-	17	18	21
	16	-	16	16	-	-	-	-	-		17	18	21
	17	17	17	17	17	17	17	17	17	17		-	-
	18	18	18	18	18	18	18	18	18	18	-		-
	21	21	21	21	21	21	21	21	21	21	-	-	

		1	4	8	9	10	12	13	15	16	17	18	21
	1		1	-	-	-	-	-	-	-	17	18	21
	4	1		-	9	10	12	13	15	16	17	18	21
	8	-	-		8	10	8	13	15	16	17	18	21
	9	-	9	8		-	-	-	-	-	17	18	21
	10	-	10	10	-		-	-	-	-	17	18	21
18	12	-	12	8	-	-		-	-	-	17	18	21
	13	-	13	13	-	-	-		-	-	17	18	21
	15	-	15	15	-	-	-	-		-	17	-	-
	16	-	16	16	-	-	-	-	-		17	18	21
	17	17	17	17	17	17	17	17	17	17		-	-
	18	18	18	18	18	18	18	18	-	18	-		-
	21	21	21	21	21	21	21	21	-	21	-	-	

Appendix 2.6.

Differences detected by the confidence intervals estimated from the logarithmic function for plant height. Number in the table are the number of biotypes that had a statistically greater value. B indicates differences detected in some harvests, but not throughout the experiment. Biotype number is the accession number presented in Table 2.1.

HARVEST	BIOTYPE	HEIGHT											
		BIOTYPE											
		1	4	8	9	10	12	13	15	16	17	18	21
GENERAL	1		B	1	-	B	-	B	-	-	B	-	-
	4	B		B	B	B	B	-	B	B	B	B	B
	8	1	B		9	B	B	--	B	B	17	18	21
	9	-	B	9		B	-	B	-	-	B	-	-
	10	B	B	B	B		-	B	-	B	17	B	B
	12	-	B	B	-	-		B	-	-	B	B	-
	13	B	-	-	B	B	B		B	B	17	18	B
	15	-	B	B	-	-	-	B		-	B	B	-
	16	-	B	B	-	B	-	B	-		B	-	-
	17	B	B	17	B	17	B	17	B	B		-	B
	18	-	B	18	-	B	B	18	B	-	-		B
	21	-	B	21	-	B	-	B	-	-	B	B	
		1	4	8	9	10	12	13	15	16	17	18	21
5	1		-	1	-	1	-	-	-	-	-	-	-
	4	-		-	-	-	-	-	-	-	-	-	-
	8	1	-		9	-	-	--	-	-	17	18	21
	9	-	-	9		9	-	-	-	-	-	-	-
	10	1	-	-	9		-	-	-	16	17	18	21
	12	-	-	-	-	-		-	-	-	17	-	-
	13	-	-	-	-	-	-		-	-	17	18	-
	15	-	-	-	-	-	-	-		-	17	-	-
	16	-	-	-	-	16	-	-	-		-	-	-
	17	-	-	17	-	17	17	17	17	-		-	-
	18	-	-	18	-	18	-	18	-	-	-		-
	21	-	-	21	-	21	-	-	-	-	-	-	

		1	4	8	9	10	12	13	15	16	17	18	21
	1		-	1	-	1	-	1	-	-	-	-	-
	4	-		-	-	-	-	-	-	-	17	18	-
	8	1	-		9	-	-	--	-	-	17	18	21
	9	-	-	9		9	-	9	-	-	-	-	-
	10	1	-	-	9		-	9	-	16	17	18	21
6	12	-	-	-	-	-		-	-	-	17	-	-
	13	1	-	-	9	-	-		-	16	17	18	-
	15	-	-	-	-	-	-	-		-	17	18	-
	16	-	-	-	-	16	-	16	-		-	-	-
	17	-	17	17	-	17	17	17	17	-		-	-
	18	-	18	18	-	18	-	18	18	-	-		-
	21	-	-	21	-	21	-	-	-	-	-	-	

		1	4	8	9	10	12	13	15	16	17	18	21
	1		1	1	-	1	-	1	-	-	-	-	-
	4	1		-	-	-	-	-	-	16	17	18	21
	8	1	-		9	-	-	--	-	16	17	18	21
	9	-	-	9		9	-	9	-	-	17	-	-
	10	1	-	-	9		-	-	-	16	17	18	21
7	12	-	-	-	-	-		-	-	-	17	18	-
	13	1	-	-	9	-	-		-	16	17	18	-
	15	-	-	-	-	-	-	-		-	17	18	-
	16	-	16	16	-	16	-	16	-		17	-	-
	17	-	17	17	17	17	17	17	17	17		-	17
	18	-	18	18	-	18	18	18	18	-	-		-
	21	-	21	21	-	21	-	-	-	-	17	-	

		1	4	8	9	10	12	13	15	16	17	18	21
	1		1	1	-	1	-	1	-	-	17	-	-
	4	1		-	9	-	12	-	15	16	17	18	21
	8	1	-		9	-	-	--	15	16	17	18	21
	9	-	9	9		9	-	9	-	-	17	-	-
	10	1	-	-	9		-	-	-	16	17	18	21
8	12	-	12	-	-	-		12	-	-	17	18	-
	13	1	-	-	9	-	12		15	16	17	18	-
	15	-	15	15	-	-	-	15		-	17	18	-
	16	-	16	16	-	16	-	16	-		17	-	-
	17	17	17	17	17	17	17	17	17	17		-	17
	18	-	18	18	-	18	18	18	18	-	-		-
	21	-	21	21	-	21	-	-	-	-	17	-	

		1	4	8	9	10	12	13	15	16	17	18	21
9	1		1	1	-	1	-	1	-	-	17	-	-
	4	1		-	9	10	12	-	15	16	17	18	21
	8	1	-		9	-	12	--	15	16	17	18	21
	9	-	9	9		9	-	9	-	-	17	-	-
	10	1	10	-	9		-	-	-	16	17	18	21
	12	-	12	12	-	-		12	-	-	17	18	-
	13	1	-	-	9	-	12		15	16	17	18	-
	15	-	15	15	-	-	-	15		-	17	18	-
	16	-	16	16	-	16	-	16	-		17	-	-
	17	17	17	17	17	17	17	17	17	17		-	17
	18	-	18	18	-	18	18	18	18	-	-		-
	21	-	21	21	-	21	-	-	-	-	17	-	
		1	4	8	9	10	12	13	15	16	17	18	21
10	1		1	1	-	1	-	1	-	-	17	-	-
	4	1		-	9	10	12	-	15	16	17	18	21
	8	1	-		9	-	12	--	15	16	17	18	21
	9	-	9	9		9	-	9	-	-	17	-	-
	10	1	10	-	9		12	-	-	16	17	18	-
	12	-	12	12	-	12		12	-	-	17	18	-
	13	1	-	-	9	-	12		15	16	17	18	-
	15	-	15	15	-	-	-	15		-	17	18	-
	16	-	16	16	-	16	-	16	-		17	-	-
	17	17	17	17	17	17	17	17	17	17		-	17
	18	-	18	18	-	18	18	18	18	-	-		18
	21	-	21	21	-	-	-	-	-	-	17	18	
		1	4	8	9	10	12	13	15	16	17	18	21
11	1		1	1	-	1	-	1	-	-	17	-	-
	4	1		8	9	10	12	-	15	16	17	18	21
	8	1	8		9	-	12	--	15	16	17	18	21
	9	-	9	9		-	-	9	-	-	17	-	-
	10	1	10	-	-		12	-	-	16	17	18	-
	12	-	12	12	-	12		12	-	-	17	18	-
	13	1	-	-	9	-	12		15	16	17	18	-
	15	-	15	15	-	-	-	15		-	17	18	-
	16	-	16	16	-	16	-	16	-		17	-	-
	17	17	17	17	17	17	17	17	17	17		-	17
	18	-	18	18	-	18	18	18	18	-	-		18
	21	-	21	21	-	-	-	-	-	-	17	18	

		1	4	8	9	10	12	13	15	16	17	18	21
12	1		1	1	-	1	-	1	-	-	17	-	-
	4	1		8	9	10	12	-	15	16	17	18	21
	8	1	8		9	10	12	--	15	16	17	18	21
	9	-	9	10		-	-	9	-	-	17	-	-
	10	1	10	8	-		12	-	-	16	17	18	-
	12	-	12	12	-	12		12	-	-	17	18	-
	13	1	-	-	9	-	12		15	16	17	18	-
	15	-	15	15	-	-	-	15		-	17	-	-
	16	-	16	16	-	16	-	16	-		17	-	-
	17	17	17	17	17	17	17	17	17	17		-	17
	18	-	18	18	-	18	18	18	-	-	-		18
	21	-	21	21	-	-	-	-	-	-	17	18	

		1	4	8	9	10	12	13	15	16	17	18	21
13	1		1	1	-	-	-	1	-	-	17	-	-
	4	1		8	9	10	12	-	15	16	17	18	21
	8	1	8		9	10	12	--	15	16	17	18	21
	9	-	9	10		-	-	9	-	-	17	-	-
	10	-	10	8	-		12	-	-	16	17	18	-
	12	-	12	12	-	12		12	-	-	17	-	-
	13	1	-	-	9	-	12		15	16	17	18	-
	15	-	15	15	-	-	-	15		-	17	18	-
	16	-	16	16	-	16	-	16	-		17	-	-
	17	17	17	17	17	17	17	17	17	17		-	17
	18	-	18	18	-	18	-	18	18	-	-		18
	21	-	21	21	-	-	-	-	-	-	17	18	

		1	4	8	9	10	12	13	15	16	17	18	21
14	1		1	1	-	-	-	1	-	-	17	-	-
	4	1		8	9	10	12	-	15	16	17	18	21
	8	1	8		9	10	12	--	15	16	17	18	21
	9	-	9	10		-	-	9	-	-	17	-	-
	10	-	10	8	-		12	-	-	-	17	18	-
	12	-	12	12	-	12		12	-	-	17	-	-
	13	1	-	-	9	-	12		15	16	17	18	-
	15	-	15	15	-	-	-	15		-	17	18	-
	16	-	16	16	-	-	-	16	-		-	-	-
	17	17	17	17	17	17	17	17	17	-		-	17
	18	-	18	18	-	18	-	18	18	-	-		18
	21	-	21	21	-	-	-	-	-	-	17	18	

		1	4	8	9	10	12	13	15	16	17	18	21
	1		1	1	-	-	-	1	-	-	17	-	-
	4	1		8	9	10	12	-	15	16	17	18	21
	8	1	8		9	10	12	--	15	16	17	18	21
	9	-	9	10		-	-	9	-	-	17	-	-
	10	-	10	8	-		12	-	-	-	17	-	-
15	12	-	12	12	-	12		12	-	-	17	-	-
	13	1	-		9	-	12		15	16	17	18	-
	15	-	15	15	-	-	-	15		-	17	18	-
	16	-	16	16	-	-	-	16	-		-	-	-
	17	17	17	17	17	17	17	17	17	-		-	17
	18	-	18	18	-	-	-	18	18	-	-		-
	21	-	21	21	-	-	-	-	-	-	17	-	
		1	4	8	9	10	12	13	15	16	17	18	21
	1		1	1	-	-	-	1	-	-	-	-	-
	4	1		8	9	10	12	-	15	16	17	18	21
	8	1	8		9	10	12	--	15	16	17	18	21
	9	-	9	10		-	-	9	-	-	17	-	-
	10	-	10	8	-		12	-	-	-	17	-	-
16	12	-	12	12	-	12		12	-	-	17	-	-
	13	1	-	-	9	-	12		15	16	17	18	-
	15	-	15	15	-	-	-	15		-	17	18	-
	16	-	16	16	-	-	-	16	-		-	-	-
	17	-	17	17	17	17	17	17	17	-		-	17
	18	-	18	18	-	-	-	18	18	-	-		-
	21	-	21	21	-	-	-	-	-	-	17	-	
		1	4	8	9	10	12	13	15	16	17	18	21
	1		1	1	-	-	-	1	-	-	-	-	-
	4	1		8	9	10	12	-	15	16	17	18	21
	8	1	8		9	10	12	--	15	16	17	18	21
	9	-	9	10		-	-	9	-	-	17	-	-
	10	-	10	8	-		12	-	-	-	17	-	-
17	12	-	12	12	-	12		12	-	-	17	-	-
	13	1	-	-	9	-	12		15	16	17	18	-
	15	-	15	15	-	-	-	15		-	-	18	-
	16	-	16	16	-	-	-	16	-		-	-	-
	17	-	17	17	17	17	17	17	-	-		-	17
	18	-	18	18	-	-	-	18	18	-	-		-
	21	-	21	21	-	-	-	-	-	-	17	-	

		1	4	8	9	10	12	13	15	16	17	18	21
	1		1	1	-	-	-	1	-	-	-	-	-
	4	1		8	9	10	12	-	15	16	17	18	21
	8	1	8		9	10	12	--	15	16	17	18	21
	9	-	9	10		-	-	9	-	-	17	-	-
	10	-	10	8	-		12	-	-	-	17	-	-
	12	-	12	12	-	12		12	-	-	-	-	-
18	13	1	-	-	9	-	12		15	16	17	18	-
	15	-	15	15	-	-	-	15		-	-	18	-
	16	-	16	16	-	-	-	16	-		-	-	-
	17	-	17	17	17	17	-	17	-	-		-	17
	18	-	18	18	-	-	-	18	18	-	-		-
	21	-	21	21	-	-	-	-	-	-	17	-	

CHAPTER 3

WILD PROSO MILLET (*Panicum miliaceum* L.) BIOTYPES: COMPETITION AND NUTRIENT UPTAKE

ABSTRACT

Experiments were carried out to compare the competitive ability of Canada-Rosemount black seeded and Colorado-Weld County tan seeded proso millet biotypes. Addition series experiments were performed using corn and sugarbeet in competition with three densities of proso millet (1, 3, and 6 plants per plot). Proso millet was planted with the crop and 14 days after the crop. Corn plants were more affected by competition with the Canada biotype than the Colorado biotype. Corn leaf area was the only parameter reduced by the Colorado biotype competition. Proso millet shoot dry weight per pot reduced an average of 47% by corn competition. Sugarbeet dry weight was reduced the same by both proso millet biotypes. Sugarbeet leaf area and shoot dry weight were reduced 38% and 41%, respectively. The Colorado biotype's shoot dry weight was reduced by the interference of sugarbeet plants. The effect

of sugarbeet competition from the Canada biotypes was verified only when proso millet was planted 14 days after the crop. The replacement series design indicated that the Canada-Rosemount black seeded was more competitive than Colorado-Weld County tan biotype. No significant differences were observed in total nutrient uptake between the biotypes; however, differences were detected between root and shoot nutrient accumulation.

INTRODUCTION

Panicum miliaceum L, proso millet, was first reported as a weed in the United States in the early 1970s (Strand et al. 1973, Doersch and Harvey 1979, Harvey 1979, Cavers and Bough 1985, Westra et al 1990). The Weed Science Society of America (1992) considers wild proso millet a weed problem in corn, sugarbeets, wheat, and soybean. Corn yield losses have been estimated at more than 2 million dollars annually in Colorado and more than 50 million dollars annually in the United States and Canada (Westra 1997[1]).

[1] Westra, P. 1997. Personal communication. Colorado State University, Ft. Collins, CO 80523

Corn yield losses from 13% to 22% were observed in field experiments when corn was in competition with wild proso millet at a density of 10 pl/m^2 (Wilson and Westra 1991). Wilson (1993), studying wild proso millet interference in dry beans, found yield reductions between 12 and 31% at a density of 10 pl/m^2. Triazine-resistant *Brassica napus* seed number and shoot dry weight were inhibited by 400 plants m^{-2} and height was reduced and flowering delayed at 600 plants m^{-2} (Miller and Callihan 1995).

The influence of one plant on another can be examined by considering plant density, proportional relationships among the species in an association, and spatial arrangement of individual plants (Cousens 1991, Harper 1994, Radosevich 1987, Radosevich et al. 1997). To study plant competition several methods have been developed: (i) additive experiments, (ii) substitutive experiments, (iii) systematic methods, and (iv) neighborhood experiments (Harper 1994, Radosevich 1987, Radosevich et al. 1997).

Even though the additive approach is the most common design for weed-crop competition studies (Radosevich 1987, Radosevich et al. 1997), it has been criticized because it does not separate the effect of density and proportion and does not permit the separation of intra and inter-specific competition (Harper 1994, Rejmanek et al. 1989, Radosevich 1987, Radosevich et al. 1997). Additive experiments maintain a constant density of one species while the density of the second species is varied (Cousens 1991, Cudney et al. 1989, Harper 1994, Radosevich 1987, Radosevich et al. 1997).

Substitutive designs address most of the problems of additive experiments (Harper 1994). Replacement series experiments, included among the substitutive designs, vary the proportion of the two species in mixture while the total plant density remains constant (Cousens 1991, Harper 1994, Rejmanek et al. 1989, Radosevich 1987, Radosevich et al. 1997).

Proso millet is classified mainly self-pollinated (Popov 1947) but biotypes have shown variability (Yashvskii 1974, Carpenter and Hopen 1985, Moore and Cavers 1985, Bough et al. 1986, McCammy and Cavers 1986, McCammy et al. 1986, Warnick and Thompson 1987, Colosi et al. 1988, Eberlein et al. 1990, Swanton and Chandler 1990, Khan et al. 1996).

The objective of this research was to compare competitive ability between two wild proso millet biotypes using additive, replacement series, and nutrient uptake experiments.

MATERIALS AND METHODS

Greenhouse experiments were conducted using two wild proso millet (*Panicum miliaceum* L.) biotypes: Canada-Rosemount black seeded and Colorado-Weld County tan seeded. Biotypes were chosen due to different growth patterns observed in Chapter 2. Seeds were obtained from the seed collection of the Colorado State University Weed Science Laboratory.

Additive experiments

A sethoxydim resistant corn variety DK 493 SR and a glyphosate resistant sugarbeet variety HM 1605 RR were used. Wild proso millet biotypes and crop seeds were germinated in germination boxes on wetted blotting paper maintained in light at room temperature (26 ± 2°C).

Germinated weed and crop seeds with 0.1 to 0.3 mm radicles were transplanted to plastic pots 2 days after incubation (DAI). Each pot (24cm diameter by 25cm deep) was filled with Sunshine® potting mix #1 (Sun Gro Horticulture, Inc., 15831 N.E. 8th Street, Bellevue, Washington 98008). Each pot was considered an experimental unit. One crop plant per pot was left to grow with three densities of wild proso millet: 1, 3, and 6 plants. Pots were fertilized every two weeks with 1.2 g of 15-30-15 Miracle-Gro® (Scotts Miracle-Gro Products, Inc., P.O. Box 888, Port Washington, New York 11050) and watered once a day. Experiments were conducted in the greenhouse with 16 h days provided by natural and artificial lightening. Greenhouse temperatures were maintained at 21 to 24°C during the day and 18 to 21°C at night.

Experiments were conducted in May 1999 in a randomized block with four replications. Experiments were repeated in June 1999. Plants were harvested 56 DAI and separated into: leaves and stems in corn; leaf, stem, and root in sugarbeet; and shoot (leaves, stem, and panicle) for proso millet biotypes. Plant height and number of leaves per plant were measured in corn, leaf area and number of leaves per plant in sugarbeet, and number of tillers in proso millet. Leaf area was determined with a leaf area meter (Licor model 3100. Li-cor, Inc., Lincoln, NE, 68583). Dry weight was obtained by placing fresh materials into paper bags and drying at 65°C for 72 h.

Data were analyzed using the SAS programming software (SAS institute 1989). Bartlett's test for homogeneity of experiment variances was performed to determine if experiments could be combined (Steel et al. 1997). Fisher's protected LSD test (P = 0.05) was used to compare means values.

Replacement series

Seed of both biotypes were germinated, as in additive experiments and transplanted into plastic pots (24cm diameter by 25cm depth filled with Sunshine® potting mix #1. Greenhouse conditions were as previously described.

A randomized complete block experimental design with four replications (four pots) was used. Treatments consisted of six ratios of wild proso millet biotypes, including two monocultures and four mixtures (10:0 8:2, 6:4, 4:6, 2:8, and 0:10). Plants were harvested at the soil surface 42 DAI. Leaf area and shoot dry weight were determined as previously.

Analysis of variance was performed as in additive experiments. Replacement series diagrams were constructed from shoot dry weight and leaf area data against weed proportions. Results were analyzed using descriptive models (Harper 1997).

Relative crowding coefficient (RCC) 42 DAI was calculated following the equation:

RCC = (mean yield per plant **a** in mixture / mean yield per plant of **b** in mixture) / (mean yield per plant **a** in monoculture / mean yield per plant **b** in monoculture) (3.1)

where **a** is the Canada-Rosemount black seeded and **b** is the Colorado-Weld County tan seeded biotype.

Relative yield (RY) was estimated for each biotype using the eqautiion:

RY = (yield in mixture / yield in monoculture) (3.2)

Relative Yield Total (RYT) and aggressivity (A) were determined according to the equations:

$$RYT = RY_a + RY_b \quad (3.3)$$

$$A = RY_a - RY_b \quad (3.4)$$

Nutrient uptake experiment

Five seeds of proso millet were planted in standard plastic pots (ITML Inc., Brantford, Ontario, Canada N3T 5M8). Each pot (15cm diameter by 14cm depth) was filled with Sakrete® washed silica sand (US Mix Products Co, Denver, Colorado, 80223) and thinned to one plant per pot. This was considered an experimental unit. Pots were fertilized every other day with nutrient solution (Hoagland and Arnon 1950) at a rate of 10ml pot^{-1} during the first 7 days increasing by 10ml every week. Water stress was avoided by supplemental watering once a day before nutrient application.

Plants were harvested 42 days after planting (DAP) by cutting the stems at soil level and dissecting plants into roots and shoot (leaves, stem and panicle). Roots were cleaned in a sieve with running water. Plant height and number of tillers were recorded. Dry weight was determined as previously.

The experimental design was a randomized complete block with six replications. For nutrient analysis, two plants were combined to make one sample. The experiment was repeated once over time. Nutrient concentrations were determined in root and shoots.

Nutrient analysis was performed by the CSU Soil, Water and Plant Testing Laboratory[2]. For N, samples were analyzed by dry combustion in a Leco 1000 CHN furnace. Plant samples were digested with nitric and perchloric acid before analysis for P, K, Ca, Mg, Fe, Mn, Zn, Cu, Bo, Na, and S.

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[2]Colorado State University, Fort Collins, CO 80523-1120

The elemental determinations were made using a Thermo Solutions IRIS Advantage Axial Inductively Coupled Plasma Atomic Absorption. Nitrates were extracted with 2% acetic acid and determination was made by calorimetric citrate determination.

Analysis of variance and Bartlett's test for homogeneity of variances from replicated experiments were performed. Mean values were compared using Fisher's protected LSD (P = 0.05) (Steel et al. 1997).

RESULTS AND DISCUSSION

Bartlett's test of all data indicated that experiment variances were not different and that experiments could be combined (Steel et al. 1997).

Corn competition

Results obtained in these experiments are presented in Tables 3.1 to 3.9 Canada-Rosemount biotype interferes more with corn than the Colorado biotype. Corn plants in competition with the Canada-Rosemount biotype were shorter than plants that grew in competition with the Colorado biotype. A similar trend was verified by number of leaves/plant and dry weight (Table 3.1). Corn plants in competition with the Colorado biotype did not differ from the results observed for corn plants grown without competition, except in leaf dry weight where corn plants were reduced about 8%. Wilson and Westra (1991) also studied the effect of wild proso millet density in corn. In their field experiments, densities of 10 and 20 plants m^{-2} reduced corn yield 13 and 38%, depending on experiment and location. Proso millet biotypes reduced total corn shoot dry weight in average 14% (Table 3.1). Scholes et al. (1995) in competition studies with corn and velvetleaf (*Abutilon theophrasti*) determined a maximum yield reduction of 32.1% due to velvetleaf competition with a reduction of 4.4% per each velvetleaf plant. Afentouli and Eletftheorohorinos (1999) working with different biotypes of carnarygrass (*Phalaris spp*) did not observe differences in competitive ability among biotypes in field competition studies with wheat. Warnick and Thompson (1987) studied differential competition in five weedy biotypes of proso millet and verified that crop-like biotypes, Russel County white seeded and Russel County golden seeded, had greater competitive ability than other crop and weedy biotypes.

Corn interference on wild proso millet is presented in Tables 3.2 to 3.9. In general, proso millet, planted at the same time as corn, showed high values than later plantings

(Table 3.2). This is explained because proso millet planted later avoided the effect of competition in early growth. The effects of density and competition were predictable on dry weights and tiller numbers (Tables 3.3 and 3.4). At high densities, proso millet biomass and tiller numbers were greater and lower per pot and per plant, respectively. When proso millet suffered corn interference, biomass and number of tillers were even lower. Although significant differences were detected for the interactions, biotypes did not differ in shoot dry weight per plot (Table 3.5). Proso millet shoot dry weight per plot was reduced 36 and 57%, respectively for Colorado and Canada biotypes, when corn and proso millet were planted at the same time (Table 3.6). Proso millet planted 14 days after corn showed no significant differences due to corn competition.

Proso millet shoot dry weight per plant was reduced by the competition with corn, and by proso millet density (Table 3.7). The Colorado biotype accumulated more shoot biomass per plant without competition than the black seeded biotype in the same situation. This result agrees with previous results obtained in both experiments of growth analysis (Chapters 2). The biomass accumulation per plant was greater at low densities than at higher densities when corn was not present.

Total tiller number per plot depended on the biotype and competition. Tiller number per plot was highest in early planting without corn competition (Tables 3.8 and 3.9). The Colorado biotype had more tillers per plant than the Canada biotype, independent of planting date.

Sugarbeet competition

Results from competition experiments between wild proso millet biotypes with sugarbeet plants are presented in Tables 3.10 to 3.19. Parameters analyzed to evaluate sugarbeet growth were not significantly different for biotypes, even though differences were observed in sugarbeet when compared with plants grown alone (Table 3.10). On average sugarbeet plants had 10 leaves with a leaf area of 839.50cm^2 and accumulated on average 6.42g of total dry weight when plants were in competition. Sugarbeet plants, grown without proso millet competition, had 3 more leaves and increased leaf area by 575.63cm^2. Total biomass accumulation in plants growing without competition was 1.6 times greater than plants stressed by competition. Schweizer (1973) using a polynomial regressions determined root losses due to competition. A linear model was the best predictor of sugarbeet losses due to kochia (*Kochia* *scoparia* (L.) Schrader) competition with plant densities of 20 plants or less per 30.5m of row. In this model one kochia plant reduced root yield 1.81%.

Excluding total shoot dry weight accumulated per pot by proso millet biotypes, number of tillers and shoot dry weight per plant had a significant ($P < 0.05$) interaction of the four factors (biotype, density, planting date, and competition) (Tables 3.11 to 3.14). The Canada biotype planted with sugarbeet did not differ in dry weight accumulation due to competition; on the other hand differences were verified for the Colorado biotype (Table 3.11). When proso millet was planted 14 days later, The Canada biotype's shoot dry weight was reduced by sugarbeet competition but the Colorado biotype's was not.

Proso millet shoot biomass per plant decreased with increasing density (Table 3.12). The Canada biotype shoot dry weight per plant did not differ due to sugabeet competition. When in competition with sugarbeet, the Canada biotype accumulated in average 58% more shoot dry weight than the Colorado biotype. Sugarbeet competition was verified for Colorado shoot dry weight per plant.

At six plants per plot without competition, tiller number/pot was higher for the Colorado biotype (30) than the Canada biotype (20). Tiller number per pot was reduced

80% due to competition effects (Table 3.13). Tiller number per plant was higher at the lowest density (Table 3.14).

Response to plant density, planting date, and competition was predictable. Higher shoot dry weight and tiller number per pot were obtained at high densities for proso millet without competition planted at same time as sugarbeet (Tables 3.15 to 3.17) Sugarbeet competition reduced tiller number and shoot dry weight of proso millet biotypes, except for tiller number per plant (Table 3.17). Shoot dry weight did not differ between biotypes due to sugarbeet competition; however, tiller number did.

Replacement series

Replacement series experiments are designed to compare biomass yields in mixtures with monoculture yields (Radosevich 1987). Harper (1994) described four models that explain the effect of one species on another in replacement series experiments. Diagrams of the relation of both biotypes are presented in Figure 3.1. Leaf area and shoot dry weight showed different responses. The effect of Canada-Rosemount black seeded biotype on Colorado Weld County tan seeded biotype was greater than plants of tan seeded biotype competing against plants of the same biotype. The reverse was also true. The tan seeded biotype had less of an effect on the black seeded biotype than when plants of black seeded biotype compete against the same. It means that these biotypes used the same resources but responded differently.

Relative yields (RY) for both species were used to calculate relative yield total (RYT) and aggressivity (A) for both biotypes (Tables 3.19 and 3.20). The Colorado biotype had lower values of relative yield for leaf area and shoot dry weight than Canada biotype. RYT describes the mutual relationship of species pairs (Harper 1994). Values close to 1 imply that the two species are placing the same demands on the same resources; values lower than one indicate that there is an antagonism relationship, and values higher than 1 indicate no competition between species. The result obtained for these wild proso millet biotypes are close to 1 and it implies the existence of competition between biotypes (Table 3.19). Afentouli and Eleftherohorinos (1999) evaluating five canarygrass biotypes, in a factorial design experiment, did not find a difference in competitive ability among biotypes. This could be explained due to the crop that was used to verify the competitive ability among biotypes. Similar results was verified in this study when sugarbeet was chosen to compete with proso millet; however, with corn competition differences could be detected.

To define the most competitive biotype, aggressivity and relative crowding coefficient (RCC) were calculated (Table 3.20). Aggressivity and RCC were higher for the Canada-Rosemount black seeded biotype compared to the Colorado-Weld County tan seeded biotype. At the proportion of 8 plants of black seeded and 2 plants of tan seeded, aggressivity measured greater than 0.70 compared to 0.26 for the opposite situation. RCC in the same proportion suggest a higher aggressiveness for the black seeded biotype. The results observed in the replacement series experiment, confirmed the superior competitive ability of Canada-Rosemount black seeded biotype detected in the corn competition experiment. If this study was just conducted with sugarbeet competition, the suggested conclusion should be that biotypes did not have different competitive ability: however, it is not true. Including other crop and other experimental design, differences in competitive ability could be detected. To study differences in competitive ability the best procedure should be test the competition between weed and crop and between weed and weed.

Nutrient uptake

Radosevich et al. (1997) stated that plant growth and competition are influence by resources and conditions. Nutrients are considered consumable resources. According to Di Tomasso (1995) weeds accumulate more nutrients than crops. Biotypes extracting more nutrients are better competitors than biotypes extracting less. Wild proso millet nutrient uptake is presented in Table 3.21.

Biotypes differed in patterns of nutrient accumulation except K, P, Mg, and Mn. Rodrigues et al. (1990) reported no differeces in nutrient accumulation by two commercial cultivars. In their research, evaluations were made in field at different periods during the entire growth season. With exception of Ca, Mg, and Mn concentration in shoots, the nutrients studied decreased in concentration from emergence to maturity. Proso millet biotypes concentrated more N, P, Ca, Mg, and B in shoots than in roots. Canada-Rosemount black seeded biotype had higher concentrations of K, Ca, Mg, Zn, and B in shoot than Colorado biotype. No differences in N, P, SO4-S, Fe, Mn, Cu, and Na concentration in shoot were detected between biotypes. The only concentration higher in Colorado biotype shoot dry weight was NO_3-N. Different patterns of accumulation were also observed in roots between biotypes. The Canada biotype accumulated higher levels of Ca and SO_4-S compared to Colorado biotype.

Biotypes did not differ in dry matter accumulation. Canada and Colorado biotypes accumulated 1.65 and 1.69g of root dry weight. Shoot dry weight accumulation was 2.13 and 2.01g, respectively for the black and tan biotypes. Nutrient uptake, calculated as function of nutrient concentration and dry matter, was not different for both biotypes. Rodrigues et al. (1990) reported that proso millet shoots accumulated 320, 30, 370, 50, 85,40, 2.3, 0.7, 0.33, and 0.15 kg ha^{-1} of N, P, K, Ca, Mg, S, Fe, Mn, Zn, and Cu, respectively at the physiological maturity. In this study wild proso millet accumulated in average 66, 6, 12, 15, and 12 kg ha-1 of N, P, K, Ca, and Mg, respectively at 42 DAP.

Studies evaluating nutrient accumulation were also done by Qasem and Hill (1995). *Senecio vulgaris* L. and *Chenopodium album* L. accumulated 27.7 and 24.9 mg g^{-1} of N, 4.8 and 4.9 mg g^{-1} of P, 35.2 and 25.4 mg g^{-1} of K, 17.9 and 15.1 mg g^{-1} of Ca, and 8.6 and 2.4 mg g^{-1} of Mg, respectively, in shoots 6 weeks after emergence.

These results and the growth analysis study (Chapter 2) could explain differences in competitive ability observed between Canada-Rosemount black seeded and Colorado-Weld tan seeded biotypes. Alone, nutrient concentration did not explain differences detected in competitive ability: however growth characteristics of the crops and weeds must also be considered. In our study, corn with different growth characteristics compared to sugarbeets suffered different competition ratios for both biotypes. It did not happen with sugarbeets due to the slower growth verified. Although differences were detected in competitive ability between the Canada and the Colorado biotypes, the competition effect in sugarbeet was not different because both biotypes were more aggressive than the crop. Competition for all factors was already initiated when sugarbeet plants began to develop.

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Table 3.1. Effect of wild proso millet competition on plant height, number of leaves, leaf dry weight, stem dry weight and total shoot dry weight of corn. Experiment was conducted under greenhouse conditions.

CORN PARAMETERS	Canada-Rosemount black seeded[1]	Colorado-Weld County tan seeded	Corn without competition
Height (cm)	59.53[B]	67.40[A]	70.31[A]
Number of leaves	10.42[B]	10.92[AB]	11.17[A]
Leaf dry weight (g)	13.47[C]	14.87[B]	16.14[A]
Stem dry weight (g)	13.11[B]	16.31[A]	17.55[A]
Total dry weight (g)	26.58[B]	31.18[A]	33.68[A]

[1] Means followed by the same superscript in a row are not significantly different by Fisher's protected LSD test at $p \leq 0.05$.

Table 3.2. Effect of planting time in the accumulation of shoot dry weight and tiller number of wild proso millet in competition with corn. Experiment was conducted under greenhouse conditions.

PARAMETERS ANALYZED	Planting period (Days)	
	0[1]	14
Total dry weight per plot	22.46^{A}	1.23^{B}
Dry weight per plant	9.19^{A}	0.40^{B}
Total tiller per plot	7.80^{A}	2.05^{B}
Tiller per plant	2.86^{A}	0.68^{B}

[1] Means followed by the same superscript in a row are not significantly different by Fisher's protected LSD test at $p \leq 0.05$.

Table 3.3. Effect of density in shoot dry weight accumulation and tiller number of wild proso millet biotypes in competition with corn. Experiment was conducted under greenhouse conditions.

PARAMETERS ANALYZED	Wild proso millet density (Plants $plot^{-1}$)		
	1[1]	3	6
Total dry weight per plot	7.39^{C}	12.47^{B}	15.67^{A}
Dry weight per plant	7.50^{A}	4.16^{B}	2.73^{C}
Total tiller per plot	2.53^{C}	5.19^{B}	7.05^{A}
Tiller per plant	2.30^{A}	1.73^{B}	1.27^{C}

[1] Means followed by the same superscript in a row are not significantly different by Fisher's protected LSD test at $p \leq 0.05$.

Table 3.4. Effect of corn competition in the accumulation of shoot dry weight and tiller number of wild proso millet biotypes. Experiment was conducted under greenhouse conditions.

PARAMETERS ANALYZED	Competition with corn	
	Without[1]	with
Total dry weight per plot	15.67^{A}	8.02^{B}
Dry weight per plant	6.72^{A}	2.87^{B}
Total tiller per plot	6.74^{A}	3.10^{B}
Tiller per plant	2.40^{A}	1.13^{B}

[1] Means followed by the same superscript in a row are not significantly different by Fisher's protected LSD test at $p \leq 0.05$.

Table 3.5. Dry weight accumulation and number of tillers of wild proso millet biotype in competition with corn. Experiment was conducted under greenhouse conditions.

PARAMETERS ANALYZED	Canada-Rosemount black seeded[1]	Colorado-Weld County tan seeded
Total dry weight per plot	11.69[A]	12.00[A]
Dry weight per plant	4.53[B]	5.06[A]
Total tiller per plot	3.54[B]	6.31[A]
Tiller per plant	1.25[B]	2.28[A]

[1] Means followed by the same superscript in a row are not significantly different by Fisher's protected LSD test at $p \leq 0.05$.

Table 3.6. Effect of planting date and competition with corn in shoot dry weight accumulation per pot by wild proso millet biotypes. Experiment was conducted under greenhouse conditions.

PLANTING DATE	Competition with corn (g plot^{-1})			
	With[1,2]	without	with	without
	Canada-Rosemount black seeded[1,2]		Colorado-Weld County tan seeded	
0	17.06[Ca]	26.69[Ba]	13.95[Da]	32.14[Aa]
14	0.56[Bb]	2.44[Ab]	0.52[Bb]	1.40[Ab]

[1] Means followed by the same capitalized superscript in a row are not significantly different by Fisher's protected LSD test at $p \leq 0.05$.
[2] Means followed by same superscript in the column are not significantly different by Fisher's protected LSD test at $p \leq 0.05$.

Table 3.7. Effect of density, planting date, and competition with corn in shoot dry weight accumulation per plant of wild proso millet biotypes. Experiment was conducted under greenhouse conditions.

PROSO MILLET DENSITY	Canada-Rosemount black seeded				Colorado-Weld County tan seeded			
	With[1,2]		without		with		without	
	0	14	0	14	0	14	0	14
1	8.20^{Ca}	0.43^{Ea}	18.11^{Ba}	0.61^{Ea}	6.70^{Da}	0.19^{Ea}	25.18^{Aa}	0.63^{Ea}
3	5.96^{Cb}	0.27^{Ea}	9.62^{Bb}	0.71^{Bb}	4.59^{Db}	0.16^{Ea}	11.41^{Ab}	0.54^{Ea}
6	4.19^{Cc}	0.07^{Da}	5.42^{Bc}	0.76^{Da}	3.56^{Cc}	0.15^{Da}	7.33^{Ac}	0.33^{Da}

[1] Means followed by the same capitalized superscript in a row are not significantly different by Fisher's protected LSD test at $p \leq 0.05$.
[2] Means followed by same superscript in the column are not significantly different by Fisher's protected LSD test at $p \leq 0.05$.

Table 3.8. Effect of corn competition on the tiller numbers of wild proso millet per plot. Experiment was conducted under greenhouse conditions..

COMPETITITON WITH CORN	Canada-Rosemount black seeded	Colorado-Weld County tan seeded
	Number of tiller per plot[1]	
With	2.29^{Bb}	3.92^{Ab}
Without	4.79^{Ba}	8.69^{Aa}

[1] Means followed by the same capitalized superscript in a row are not significantly different by Fisher's protected LSD test at p≤0.05.

Table 3.9. Effect of planting date on tiller numbers of wild proso millet biotypes in competition with corn. Experiment was conducted under greenhouse conditions.

PLANTING DATE	Canada-Rosemount black seeded[1]	Colorado-Weld County tan seeded
	Number of tiller per plot	
0	5.42[Ba]	10.17[Aa]
14	1.67[Ab]	2.44[Ab]
	Number of tiller per plant	
0	2.03[Ba]	3.68[Aa]
14	0.48[Bb]	0.88[Ab]

[1] Means followed by the same capitalized superscript in a row are not significantly different by Fisher's protected LSD test at p≤0.05.

Table 3.10. Effect of wild proso millet competition on sugarbeet leaf number, leaf dry weight, stem dry weight and total shoot dry weight of sugarbeet. Experiment was conducted under greenhouse conditions.

SUGARBEETS PARAMETERS	Canada-Rosemount black seeded[1]	Colorado-Weld County tan seeded	Sugarbeet without competition
Number of leaves	10.14^B	10.34^B	13.36^A
Leaf area (cm^2)	859.32^B	819.67^B	1408.67^A
Leaf dry weight (g)	3.61^B	3.62^B	5.65^A
Stem dry weight (g)	1.65^B	1.49^B	2.52^A
Sugarbeet root (g)	1.33^B	1.20^B	1.93^A
Total dry weight (g)	6.36^B	6.48^B	10.09^A

[1] Means followed by the same capitalized superscript in a row are not significantly different by Fisher's protected LSD test at $p \leq 0.05$.

Table 3.11. Effect of planting date and sugarbeet competition in wild proso millet shoot dry weight per pot. Experiment was conducted under greenhouse conditions.

PLANTING DATE	Canada-Rosemount black seeded		Colorado-Weld County tan seeded	
	Competition with corn		Competition with corn	
	With[1,2]	without	with	without
0	28.75[Ba]	29.24[Ba]	28.33[Ba]	33.06[Aa]
14	1.90[Bb]	3.92[Ab]	1.37[Bb]	2.24[Bb]

[1] Means followed by the same capitalized superscript in a row are not significantly different by Fisher's protected LSD test at p≤0.05.
[2] Means followed by same superscript in the column are not significantly different by Fisher's protected LSD test at p≤0.05.

Table 3.12. Effect of density, planting date, and competition with sugarbeet in shoot dry weight accumulation per plant of wild proso millet biotypes. Experiment was conducted under greenhouse conditions.

PROSO MILLET DENSITY	Canada-Rosemount black seeded				Colorado-Weld County tan seeded			
	With[1,2]		without		with		without	
	0	14	0	14	0	14	0	14
1	20.98^{Ba}	0.92^{Da}	22.19^{Ba}	0.93^{Da}	13.16^{Ca}	0.51^{Da}	29.24^{Aa}	1.04^{Da}
3	10.42^{Bb}	0.70^{Ca}	11.09^{Bb}	1.60^{Ca}	10.70^{Bb}	0.47^{Ca}	12.45^{Ab}	0.69^{Ca}
6	5.93^{Ac}	0.45^{Ba}	5.61^{Ac}	1.00^{Ba}	6.44^{Ac}	0.37^{Ba}	6.13^{Ac}	0.61^{Ba}

[1] Means followed by the same capitalized superscript in a row are not significantly different by Fisher's protected LSD test at $p \leq 0.05$.

[2] Means followed by same superscript in the column are not significantly different by Fisher's protected LSD test at $p \leq 0.05$.

Table 3.13. Effect of density, planting date, and competition with sugarbeet in total number of tiller per pot of wild proso millet biotypes. Experiment was conducted under greenhouse conditions.

PROSO MILLET DENSITY	Canada-Rosemount black seeded				Colorado-Weld County tan seeded			
	With[1,2]		without		with		without	
	0	14	0	14	0	14	0	14
1	6.25^{Bc}	1.13^{Ca}	6.63^{Bb}	1.88^{Cb}	11.63^{Ac}	1.25^{Ca}	10.13^{Ab}	3.88^{Bb}
3	11.13^{Cb}	1.25^{Ea}	8.63^{Cb}	5.38^{Aa}	19.25^{Bb}	2.88^{DEa}	21.38^{Aa}	4.75^{Db}
6	16.00^{Ca}	0.88^{Ea}	14.25^{Ca}	7.38^{Da}	29.88^{Aa}	2.75^{Ea}	21.00^{Ba}	9.38^{Da}

[1] Means followed by the same capitalized superscript in a row are not significantly different by Fisher's protected LSD test at p≤0.05.
[2] Means followed by same superscript in the column are not significantly different by Fisher's protected LSD test at p≤0.05.

Table 3.14. Effect of density, planting date, and competition with sugarbeet in number of tiller per plant of wild proso millet biotypes. Experiment was conducted under greenhouse conditions.

PROSO MILLET DENSITY	Canada-Rosemount black seeded				Colorado-Weld County tan seeded			
	with		without		with		without	
	0[1,2]	14	0	14	0	14	0	14
1	6.25^{Ca}	1.13^{Ea}	6.63^{Ca}	1.88^{DEa}	10.86^{Aa}	1.25^{Ea}	9.27^{Ba}.	2.57^{Da}
3	3.71^{Bb}	0.42^{Da}	2.88^{Bb}	1.79^{Ca}	6.19^{Ab}	0.96^{CDa}	7.13^{Ab}	1.58^{Ca}
6	2.67^{BCc}	0.15^{Fa}	2.38^{Cb}	1.23^{DEa}	4.98^{Ac}	0.46^{EFa}	3.50^{Bc}	1.56^{CDa}

[1] Means followed by the same capitalized superscript in a row are not significantly different by Fisher's protected LSD test at $p \leq 0.05$.

[2] Means followed by same superscript in the column are not significantly different by Fisher's protected LSD test at $p \leq 0.05$.

Table 3.15. Effect of density in shoot dry weight accumulation and tiller number of wild proso millet biotypes in competition with sugarbeet. Experiment was conducted under greenhouse conditions.

PARAMETERS ANALYZED	Wild proso millet density (Plants plot^{-1})		
	1*	3	6
Total dry weight per plot	10.04^{BC}	18.33^{B}	19.93^{A}
Dry weight per plant	11.12^{A}	6.01^{B}	3.32^{C}
Total tiller per plot	5.34^{C}	9.33^{B}	12.69^{A}
Tiller per plant	4.98^{A}	3.08^{B}	2.12^{C}

* Means followed by the same capitalized superscript in a row are not significantly different by Fisher's protected LSD test at p≤0.05.

Table 3.16. Effect of planting period in the accumulation of shoot dry weight and tiller number of wild proso millet growing with sugarbeet. Experiment was conducted under greenhouse conditions.

PARAMETERS ANALYZED	Planting period (Days)	
	0[1]	14
Total dry weight per plot	29.84[A]	2.36[B]
Dry weight per plant	12.86[A]	0.77[B]
Total tiller per plot	14.68[A]	3.56[B]
Tiller per plant	5.54[A]	1.25[B]

[1] Means followed by the same capitalized superscript in a row are not significantly different by Fisher's protected LSD test at p≤0.05.

Table 3.17. Effect of corn competition in shoot dry weight and tiller number of wild proso millet biotypes. Experiment was conducted under greenhouse conditions.

PARAMETERS ANALYZED	Competition with corn	
	without[1]	with
Total dry weight per plot	17.11[A]	15.09[B]
Dry weight per plant	7.72[A]	5.92[B]
Total tiller per plot	9.55[A]	8.69[B]
Tiller per plant	3.53[A]	3.25[B]

[1] Means followed by the same capitalized superscript in a row are not significantly different by Fisher's protected LSD test at $p \leq 0.05$.

Table 3.18. Dry weight accumulation by wild proso millet biotype in competition with sugarbeet. Experiment was conducted under greenhouse conditions.

PARAMETERS ANALYZED	Canada-Rosemount black seeded[1]	Colorado-Weld County tan seeded
Shoot dry weight per plot	15.95[A]	16.25[A]
Shoot dry weight per plant	6.82[A]	6.82[A]
Total tiller per plot	6.73[B]	11.51[A]
Tiller per plant	2.59[B]	4.19[A]

[1] Means followed by the same capitalized superscript in a row are not significantly different by Fisher's protected LSD test at $p \leq 0.05$.

Table 3.19. Relative yield (RY) from leaf area and shoot dry weight of wild proso millet biotypes in greenhouse replacement series experiment 42 days after planting.

SPECIES PROPORTION (%)	RY Canada	RY Colorado	RYT
	Leaf area		
80-20	0.81	0.11	0.92
60-40	0.7	0.23	0.93
40-60	0.52	0.44	0.95
20-80	0.34	0.58	0.92
	Shoot dry weight		
80-20	0.87	0.12	0.99
60-40	0.78	0.25	1.03
40-60	0.55	0.47	1.02
20-80	0.35	0.6	0.95

Table 3.20. Relative crowding coefficient (RCC) and aggressiveness (A) from leaf area and shoot dry weight of wild proso millet biotypes in greenhouse replacement series experiment 42 days after planting.

SPECIES PROPORTION (%)	Relative crowding coefficient (RCC)		Aggressiveness (A)	
	Canada-Colorado	Colorado-Canada	Canada-Colorado	Colorado-Canada
(%)	Leaf area			
80-20	7.17	0.14	0.75	-0.75
60-40	3.19	0.31	0.54	-0.54
40-60	1.17	0.85	0.08	-0.08
20-80	0.57	1.74	-0.26	0.26
	Shoot dry weight			
80-20	7.14	0.14	0.70	-0.70
60-40	3.00	0.33	0.47	-0.47
40-60	1.18	0.85	0.08	-0.08
20-80	0.59	1.70	-0.24	0.24

Table 3.21. Concentration of nutrients in shoot and root of wild proso millet biotypes 42 days after planting.

BIOTYPE	ELEMENTS												
	%		ppm										
	N	P	K	Ca	Mg	SO_4-S	Fe	Zn	Mn	Cu	B	Na	NO_3-N
	SHOOT												
Canada-Rosemount black seeded	1.73	0.16	2.82	4302.00	2756.63	431.38	40.84	15.99	18.80	5.79	15.17	133.00	25.86
Colorado Weld County tan seeded	1.78	0.17	2.50	2351.75	2408.13	203.38	41.00	11.89	13.66	5.92	11.67	93.34	64.86
	ROOT												
Canada-Rosemount black seeded	1.17	0.11	2.37	4797.63	2053.75	2154.88	112.11	39.04	15.90	32.50	7.49	2416.88	26.17
Colorado Weld County tan seeded	0.92	0.09	2.81	2948.88	2231.50	1085.00	77.41	31.08	22.15	19.82	4.98	1830.75	64.86
LSD 0.05%	0.05	0.01	0.07	281.46	72.31	183.50	3.14	1.94	2.65	0.40	0.44	102.90	12.66

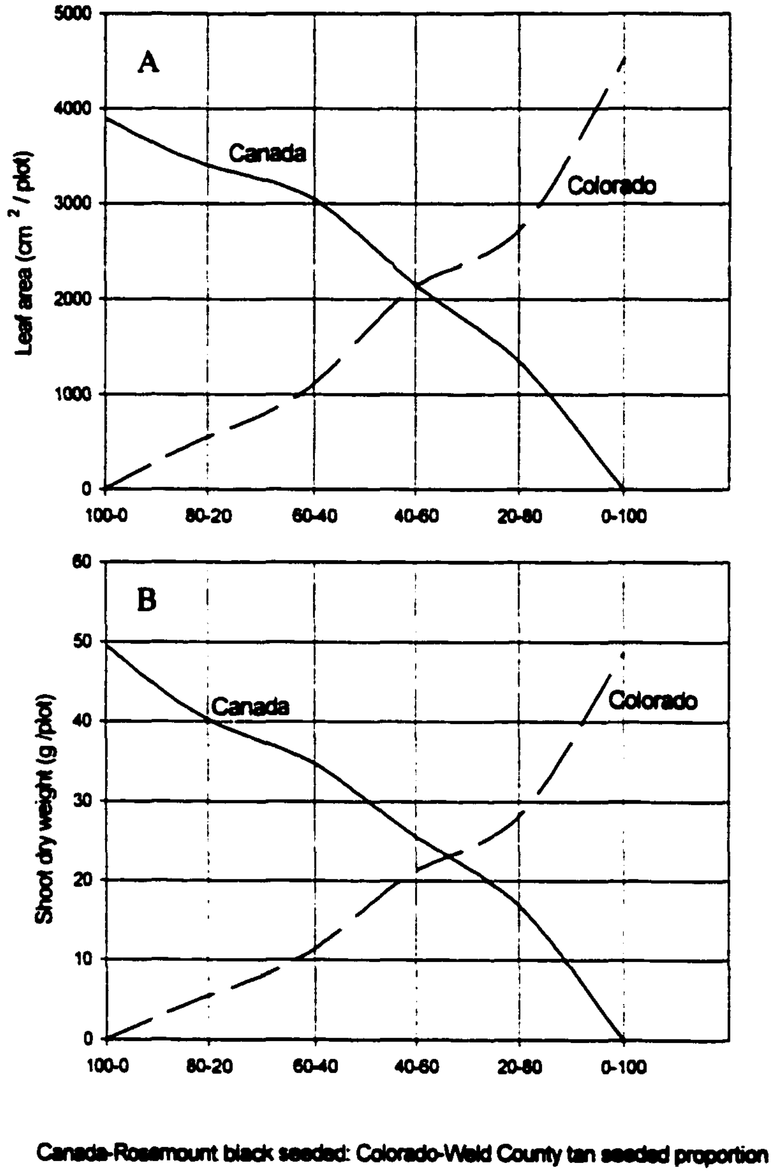

Figure 3.1. Diagrams of Canada-Rosemount black seeded:Colorado-Weld County tan seeded biotypes in replacement series. Leaf area (A) and shoot dry weight (B).

CHAPTER 4

GENETIC DIVERSITY AMONG WILD AND DOMESTIC PROSO MILLET (*Panicum miliaceum* L.) BIOTYPES

ABSTRACT

Amplified fragment length polymorphism (AFLP) was used to assess genetic diversity among three domestic and nine wild proso millets originated from the United States and Canada. A high resolution polyacrylamide gel was used to separate PCR-amplified DNA fragments and stained with silver stain. Eight primers combination detected 39 polymorphic DNA bands. Genetic distance ranged from 0.02 to 0.04. Colorado-Weld County black seeded biotype and Wyoming-Platte County were the most distinct biotypes according to level of dissimilarity. A UPGMA cluster analysis reveled two distinct groups of proso millet but without any geographic association. The cluster analysis indicated a close relation between two wild black seeded biotypes and the domestic orange biotype. AFLP's polymorphic fragments may be more related to variation within a population than variation between populations.

INTRODUCTION

Charles Darwin observed that individual members of a given species are not exactly alike, but show a great deal of variation. In 1858, he proposed that due to variation, some individuals would be more suited than others to the environment in which they were living. He concluded that the species are not fixed but that their characteristics change over time and suggested that the driving force behind this change is the number of offspring each individual leaves as a result of its fitness for the environment (Chrispeels and Sadava 1994).

According to Begon et al (1996) "genetic variation is determined by the joint action of natural selection and genetic drift". Mutation, recombination, and gene flow can generate genetic variation (Curtis 1975, Campbell 1993).

Proso millet, the major cultivated grain crop in Europe since 2000 BC (Anderson and Martin 1949, Grabouski 1971, Baltensperger 1996), is currently produced in Eastern Europe, Russia, China, India, and North America. Even though it is considered to be self-pollinated with at least 10% of cross pollination (Popov 1947), different types of proso millet have been reported (Yashovskii 1974, Bough et al. 1986, Eberlein et al. 1990, Carpenter and Hopen 1985, Khan et al. 1996, Warnick and Thompson 1987, Moore and Cavers 1985, McCamy et al. 1988, McCamy and Cavers 1988, Colossi et al. 1988, M'Ribu and Hilu 1994, Colossi and Shaal 1997, Warnick 1987). In the last 25 years, wild-proso millet, the weedy form of proso millet, has become one of the most aggressive grass weeds in North America. The crop was first introduced to Canada in the 17th century (Bough and Cavers 1987). High infestation of this weed were first reported in Minnesota and Wisconsin in the early 1970s (Strand et al. 1973, Cavers and Bough 1985, Doersch and Harvey 1979, Harvey 1979). Since this period, wild-proso millet has become a problem in different regions of the United States and Canada (Bough et al. 1986, Eberlein et al. 1990, WSSA 1992).

Hatzios (1987) suggested four areas where biotechnology offers opportunities in weed science: (i) the development and use of bioherbicides, (ii) the discovery of natural enemies as biological control agents, (iii) the development of crop tolerance to herbicides, and (iv) the use of genetically engineered microorganisms. Techniques in molecular biology have been used as tools to quantify genetic diversity and match natural enemies with the target weed. These techniques have been helping taxonomists, evolutionary biologists, and breeders to understand the relationships among populations (Nissen et al 1995).

Several types of markers have been used to assess genetic diversity. Restriction fragment length polymorphism (RFLP) developed by Botstein et al. (1980) has been used as tools for detecting polymorphism among and within species (Guttieri et al. 1992, Krugman et al. 1997). This is a laborious, expensive procedure and few loci are detected per assay (Hill et al, 1996).

Random amplified polymorphic DNA (RAPD)has also been used to detect polymorphism as well as to develop detailed genetic maps (Katzir et al. 1996, Vellekoop et al. 1996, Rowe et al. 1997). In proso millet, Colosi and Shaal (1997) found 97 RAPD genotypes (69 wild proso millet, 26 crop and feral crop weed, and 2 hybrids) among 398 individuals. In about 10% of the genotypes, suggests hybridization between wild proso millet and crop biotypes. This technique uses the polymerase chain reaction (PCR) and single oligonucleotide primer of arbitrary sequences (Hill et al (1996).

Even though many studies of wild proso millet have been developed, more work has to be done to understand the differences observed in this species. Amplified Fragment Length Polymorphism (AFLP), a new molecular technique developed by Marc Zabeau and researchers at Keygene N. V. (Zabeau 1992, Zabeau and Vos 1993, Vos et al. 1995, Blears et al. 1998) for DNA fingerprinting, is based on the existence of variations between species, varieties and cultivars (Liscum and Oeller 1999). AFLP technique includes three major steps: (i) restriction of the DNA and ligation with specific adapters, (ii) amplification of restriction fragments, and (iii) analysis of the amplified fragments by gel electrophoresis (Vos et al. 1995). The AFLP technique has advantages over RFLP and RAPD such as detection of large numbers of polymorphic fragments from a PCR reaction (VanToai et al. 1996a, 1996b).

Several experiments have been developed with this new technique. Becker et al. (1995) combined AFLP and RFLP markers in barley. Mackill et al. (1996) evaluated levels of polymorphism and genetic mapping in rice. Cho et al. (1996) also used AFLP technique for cloning and mapping of variety-specific rice genomic DNA sequences. Soybean has been evaluated by VanToai et al. (1996a, 1996b) who studied the use of AFLP to determine the contribution of parental genomes during recurrent selection. Lin et al (1996a) studied soybeans comparing RFLP, RAPD, and AFLP techniques. AFLP was used to study genetic structure between and within gene pools of wild bean (*Phaseolus vulgaris*) (Tohme et al.

1996). AFLP also has been utilized in other plant species such as *Astragalus cremnophylax* var. *cremnophylax* (Travis et al, 1996), *Miscanthus* spp. (Greef et al. 1997), *Camellia sinensis* (Paul et al. 1997), *Lactuca* spp. (Hill et al. 1996), *Populus* spp (Cervera et al. 1996), and potato (Meksem et al. 1995). Other researchers have used AFLP to understand genetic diversity within a population of bacteria (Voorrips et al. 1997; Huys et al. 1996; Lin et al. 1996b).

The purpose of this study is to evaluate the genetic diversity in wild and domestic proso millet biotypes using the AFLP technique.

MATERIALS AND METHODS

Three domestic and nine wild biotypes of proso millet (Table 2.1) were grown in a growth chamber (16 h photoperiod, 25/20°C (day/night temperature), and 50% relative humidity) for two weeks.

DNA was extracted from leaves. Leaves were ground with liquid nitrogen to obtain approximately 100 mg of fine powder. DNA was isolated with Dneasy Plant Mini Kit (Qiagen Inc, Valencia, CA 91355). The procedure precipitates cell debris and salt using a QIAshredder spin column. Purified DNA was eluted from a Dneasy spin column.

The AFLP procedure is property of KeyGene (Wageningen, The Netherlands) (Zabeau and Vos 1993). The method used was based on AFLP™ Analysis System and AFLP Starter Primer protocol provided from GibcoBRL (Life Technologies, Inc.).

Restriction digestion of the DNA was performed as described in GibcoBRL protocol. Briefly, proso millet DNA (500 ng) was restricted with 2.5 units of *Eco*R I and *Mse* I in 2 µl of *Eco*R I/*Mse* I in buffer solution (1.25 units/µl each, 10 mM Tris-HCl (pH 7.5), 50 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.1 mg/ml BSA, 50% (v/v) glycerol, 0.1% Triton® X-100). The mixture was incubated for 2 h and 30 min at 37°C. Before ligation, mixture was left 15 min at 70°C and placed on ice for 5 min. The ligation was done with 24 µl of the adapter ligation solution ((EcoR I/ Mse I adapters, 0.4 mM ATP, 10 mM Tris-HCl (pH 7.5), 10 mM Mg-acetate, 50 mM K-acetate) for 2 h at 20°C. Preamplification was made with 5 µl of template DNA (ligated DNA), 1 µl (5 units) of *Taq* DNA polymerase and 40 µl pre-amp primer mix. A 20 cycle (94°C for 30 s, 52°C for 60 s, 72°C for 60 s) PCR was performed. A dilution (1:5) using TE buffer was made with the PCR result. All reagents and solutions were obtained in the AFLP Core reagents kit from GibcoBRL.

Selective amplification was modified from the original protocol from GibcoBRL. *Eco*R I and *Mse* I (30 ng each) was mixed with 2 µl of diluted proso millet DNA, 2 µl of 10X PCR buffer (200 mM Tris-HCl (pH 8.4), 15 mM $MgCl_2$, 500 mM KCl), 0.2 µl *Taq* polymerase (5 units/µl) and brought to 20 µl of solution with distilled water. A PCR was performed using a PTC-100™ programmable Thermal Controller (MJ Research, Inc) following the protocol presented by GibcoBRL (Table 5.2). Selectively amplified fragments were mixed with an equal volume of formamide, denatured at 95°C for 5 min, and incubate on ice.

Polyacrylamide gel electrophoresis was performed in a Sequi-Gen 0.04 x 38 x 50 cm apparatus (Bio-Rad Laboratories, Richmond, CA) Plates were washed with Alcanox (Alcanox Inc.), Sequesoap 2% (Gold Technology, Inc.), and Sequestrip (Gold Technology, Inc.). Glasses were rinsed with EtOH (95%) and treated with methacryloxypropyltri methoxysilane (Sigma Chemical Co.). Five micro liters of each sample was applied onto a 5.3% polyacrilamide gel [40.56 g Urea, 12 ml 40% acrylamide, 18 ml 5X TBE (54g Tris, 55g boric acid, 40 ml 0.25 M EDTA pH 8.0)] and eletrophoresis was carried out in 1X TBE at constant 75W for 2 h and 45 min at 50°C with a PC 3000 Power Supply (Bio-Rad Laboratories, Richmount, CA).

Gels were stained after 25 min with gentle agitation in 10% acetic acid followed by three 2 min water rinse. The silver staining was performed for 30 min in a solution containing 2 g silver nitrate, 3 ml 37% formaldehyde in 2 liters water. After that gels were rinsed in water for 10 sec and developed in a solution containing 60 g of sodium carbonate (Fisher Chemical), 3 ml formaldehyde and 400 µl of sodium thiosulfate (Fisher Chemical). Once bands were visible the developing process was stopped adding the 10% glacial acetic acid solution recovered from the first step.

The data were analyzed by the UPGMA (unweighted pair-group method with arithmetical averages) of the software NTSYS-PC, version 2.02j (Exeter Software). Dendograms were created with the tree program of NTSYS. Genetic distances were calculated following the equation:

$$\mathbf{Sij} = 2\mathbf{a} / (2\mathbf{a} + \mathbf{b} + \mathbf{c}) \quad \text{(Nei and Li 1979)} \qquad (4.1)$$

where **Sij** is the similarity between two biotypes, **i** and **j**, **a** is the number of bands present in both **i** and **j** biotypes, **b** is the number of bands present in **i** biotype and **c** is the number of bands present in **j** biotype. Conversion to genetic distance (**Gdij**) was made by:

$$\mathbf{Gdij} = 1 - Sij \qquad (4.2)$$

RESULTS AND DISCUSSION

A high resolution polyacrylamide gel without radioactive isotopes is an efficient technique to visualize PCR-amplified fragments for the AFLP's amplified proso millet DNA products. Silver-staining method is also preferable to a standard radioactive method because it eliminates the necessity of working with radioactivity (see Chalhoulb et al. (1997) for details on the efficacy of silver-staining procedure).

Eight combinations of AFLP's primers produced at least 450 bands which were at times difficult to read; of these 339 clearly separated bands, 39 polymorphic DNA bands were detected (Figures 4.1 to 4.8). Vos et al. (1995) reported that the typical restriction fragments amplified by AFLP detect 50 to 100 fragmentson eletrophoresis in polyacrylamide gel. Our results averaged 56 amplified fragments per polyacrilamid gel.

The combination of primers with two selective nucleotides and three selective nucleotides separated more bands than the combination three and three selective nucleotides (Figures 4.1 and 4.2). These bands were, however, difficult to read. Proso millet has been classified as a tetraploid (2n = 36 chromosomes) (Bajaj 1981, M'Ribu and Hilu 1994) whereas *Panicum* diploids (2n = 36) are known (Purseglove 1972, Teslev 1976). Blears et al. (1998) in a review of the AFLP procedure and its application states that small genomes (10^6-10^7 base pairs) need one to two selective nucleotides on the 3'-end of each primer to detect polymorphism while more complex genomes (10^8-10^{10}bp) needs additional selective nucleotides. Among the primer combinations, Mse-CTT/EcoR-AAC and Mse-CAA/EcoR-ACT were the most informative producing on the average eight polymorphic bands (Figures 4.7 and 4.8).

Colossi and Schaal (1997) found two biotypes genetically intermediate between the wild and domestic proso millet biotypes. One of these, called Cambridge originated from Minnesota, showed less weedy characteristics than other wild biotypes studies (Eberlein et al. 1990, Westra and Callan 1990). Based on RAPD analysis and studies by Eberlein et al. (1990) and Striegel and Boldt (1981), Cambridge biotype is believed to has originated to hybridization events between wild and domestic proso millet biotypes; however, based on RAPD analysis no evidence of the hybridization was detected (Colosi and Schaal 1997)

The percent of polymorphic bands detected between Minnesota-Cambridge and Oregon biotypes calculated by the number of polymorphic bands divided by the number of monomorphic bands was 6.4% and for the black seeded biotypes it was 3.9%. The level of polymorphism detected in the present study (11%) is consistant with the usefulness of AFLP in determining intraspecific variation among proso millet biotypes.

A matrix of the presence or absence of bands was analyzed by two methods. Genetic distance was determined according to Nei and Li's (1979) and genetic similarity according to Thome et al. 1996. Table 4.1 shows the genetic similarity calculated among proso millet biotypes. The results showed that the genetic distance ranged from 0.02 to 0.04. The greatest differences were observed between Colorado-Weld County black seeded and Wyoming-Platte County brown seeded. The closest related biotypes were the tan seeded biotypes from Nebraska and Colorado.

Genetic similarities has been measured by using the equation:

$$Gs_{ij} = C_{ij} / N_{ij} \quad (4.3)$$

where **Gs** is the measurement of the genetic similarity between biotypes **i** and **j**, C_{ij} is the number of bands present in i and **j**, and N_{ij} is the total bands scored (Hill et al. 1996).

Even though the UPGMA and neighbor-joining (NJ) methods can derive more than one topology from the same data (Backeljau et al. 1996, Bruno et al. 2000), the UPGMA method was selected to draw the dendograms. Both methods consist of two steps: choosing a pair of taxa to be joined and distances from the new node to all other nodes are inferred (Bruno et al. 2000).

Analysis conducted by M'Ribu and Hilu (1994) through RAPD and UPGMA association based on the Dice (1945) algorithm of similarity, grouped proso millet according to their geographic origin. In our results (Figure 4.9), determined using the same unweighted pair group method but based on NEI72 (Nei 1972), biotypes did not group by geographic distribution. It should be pointed out that the geographic location observed in our study was limited than the scale verified in M'Ribu and Hilu (1994). To verify and contrast our results with M'Ribu and Hilu (1994), a cluster analysis was done based on the same DICE algorithms (Figure 4.10). Coefficients estimated from DICE algorithmic ranged from 0.96 to 1.0 while M'Ribu and Hilu (1994) estimated coefficients varied from 0.60 and 1.0.

In our experiments described in preceding section (Chapter 2) in which analyses of mean shoot, leaf area, and absolute growth rate (Table 2.3), plant height (Figures 2.30 and 2.33), and dry weight partitioning (Figure 2.42 and 2.45) for Canada-Rosemount and Colorado tan seeded biotypes was performed, the results showed that the seed weight (Table 2.5) and seed coat percent (Table 2.5) were not significant different. However, when comparing the competitive ability of both biotypes, differences in agressivity were detected. The Canada biotype was more aggressive than the Colorado biotype (Chapter 3). Differences were also detected in nutrient accumulation between Canada-Rosemount and Colorado tan seeded biotypes (Chapter 3). We believe that the differences observed in growth and competitive ability may be related to genetic differences observed in this experiment. Even though just 9% of polymorphic DNA fragments between both biotypes was separated by the AFLP procedure (Table 4.2).

Based on AFLP polymorphic DNA fragments analysis and the genetic distance estimates, we suggest that both

Canada-Rosemount and Colorado biotypes are more closely related to domestic biotypes than tan seeded and Ontario-Canada black seeded biotypes. It should be stated that this study was conducted using one plant developed from one seed selected from a seed population of each biotype. We suggest that the variations observed in the present study are more related to the variations that may occur within a population than the variations across populations.

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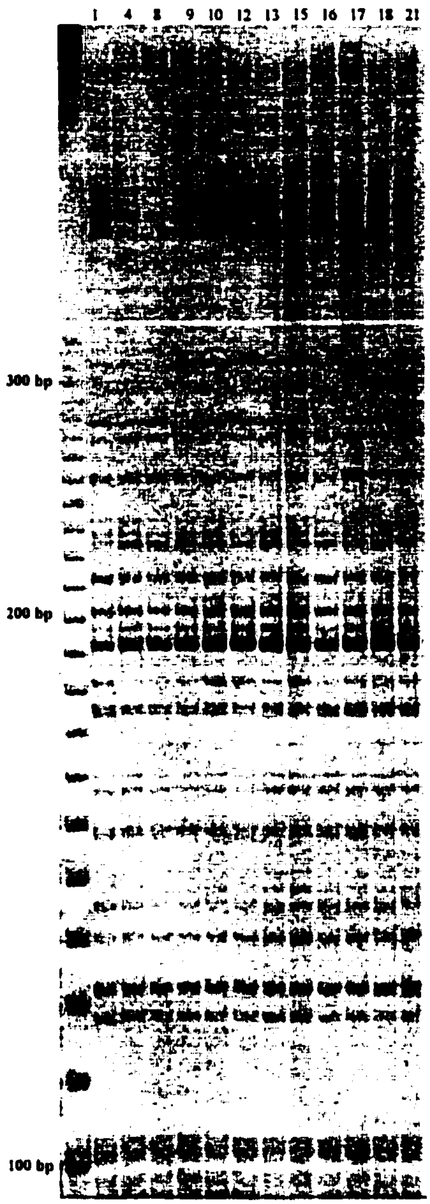
Table 4.1. Genetic similarity coefficients among proso millet biotypes estimated by Nei and Li (1979) definition of similarity.

ACCESSION NUMBER	ORIGIN	SEED COLOR	ACCESSION NUMBER											
			1	4	8	9	10	12	13	15	16	17	18	21
1	Minnesota-Cambridge	olive												
4	Canada-Rosemount	black	0.98											
8	Oregon-Grand Island	olive	0.97	0.97										
9	Ontario, Canada-Huron County	black	0.98	0.98	0.98									
10	Nebraska-Panhandle Center	tan	0.97	0.96	0.97	0.96								
12	Wyoming-Platte County	brown	0.97	0.96	0.97	0.97	0.99							
13	Colorado-Weld County	black	0.98	0.98	0.97	0.98	0.96	0.96						
15	Colorado-Weld County	tan	0.97	0.96	0.97	0.96	1.00	0.99	0.96					
16	South Dakota	brown	0.98	0.98	0.97	0.98	0.97	0.96	0.99	0.96				
17	Colorado	white	0.98	0.99	0.97	0.98	0.97	0.96	0.99	0.96	0.99			
18	Colorado	orange	0.98	1.00	0.97	0.98	0.96	0.96	0.99	0.96	0.99	0.99		
21	Colorado-Larimer	white	0.98	0.99	0.97	0.98	0.97	0.96	0.99	0.96	0.98	0.99	0.99	

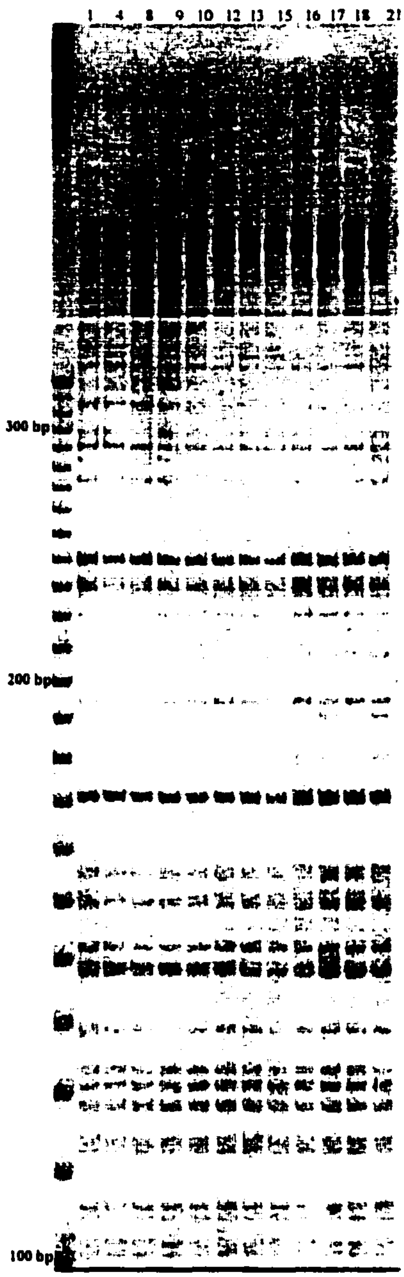
Table 4.2. Ratio of pholymorphic DNA fragments detected by AFLP procedure on domestic and wild proso millet biotypes.Nei and Li (1979) definition of similarity.

ACCESSION NUMBER	ORIGIN	SEED COLOR	ACCESSION NUMBER											
			1	4	8	9	10	12	13	15	16	17	18	21
1	Minnesota-Cambridge	olive												
4	Canada-Rosemount	black	0.04											
8	Oregon-Grand Island	olive	0.06	0.06										
9	Ontario, Canada-Huron County	black	0.05	0.04	0.05									
10	Nebraska-Panhandle Center	tan	0.07	0.09	0.07	0.08								
12	Wyoming-Platte County	brown	0.06	0.08	0.06	0.07	0.02							
13	Colorado-Weld County	black	0.05	0.03	0.06	0.05	0.07	0.09						
15	Colorado-Weld County	tan	0.07	0.09	0.06	0.08	0.00	0.02	0.08					
16	South Dakota	brown	0.05	0.04	0.07	0.05	0.07	0.08	0.02	0.08				
17	Colorado	white	0.03	0.03	0.05	0.05	0.07	0.08	0.02	0.08	0.02			
18	Colorado	orange	0.03	0.01	0.06	0.04	0.08	0.08	0.01	0.08	0.03	0.02		
21	Colorado-Larimer	white	0.03	0.02	0.06	0.05	0.07	0.08	0.02	0.08	0.04	0.02	0.02	



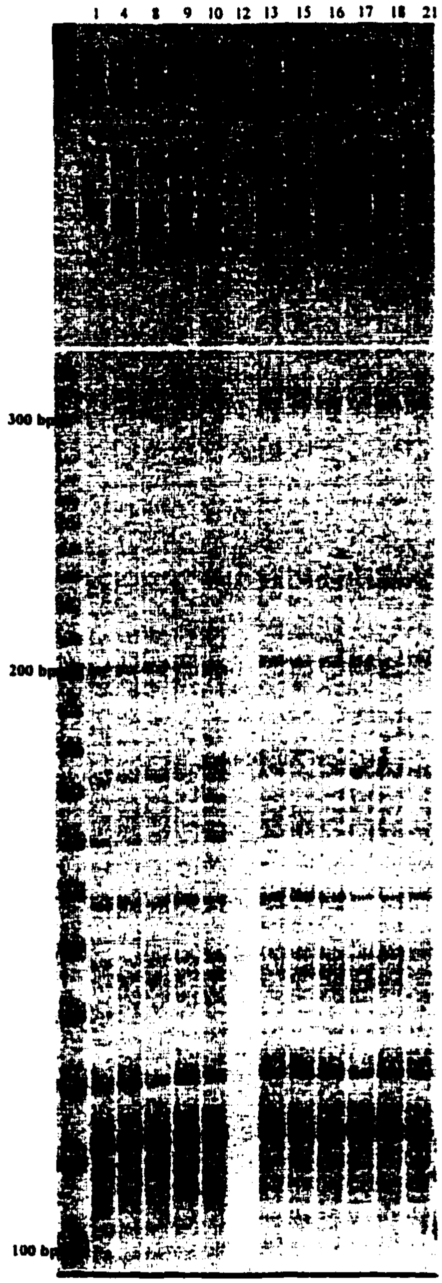
1 - Minnesota-Cambridge wild olive seeded
4 - Canada-Rosemount wild back seeded
8 - Oregon-Grand Island wild olive seeded
9 - Ontario, Canada-Huron County wild black seeded
10 - Nebraska-Panhandle Center wild tan seeded
12 - Wyoming-Platte County wild brown seeded
13 - Colorado-Weld County wild black seeded
15 - Colorado-Weld County wild tan seeded
16 - South Dakota domestic brown seeded
17 - Colorado domestic white seeded
18 - Colorado domestic orange seeded
21 - Colorado wild white seeded

Figure 4.1. AFLP pattern on twelve proso millet biotypes derived by the primer combination Mse-CAA/EcoR-TC. Numbers on gel correspond to biotypes listed on the right. DNA marker separated in 10bp is presented in the first column.



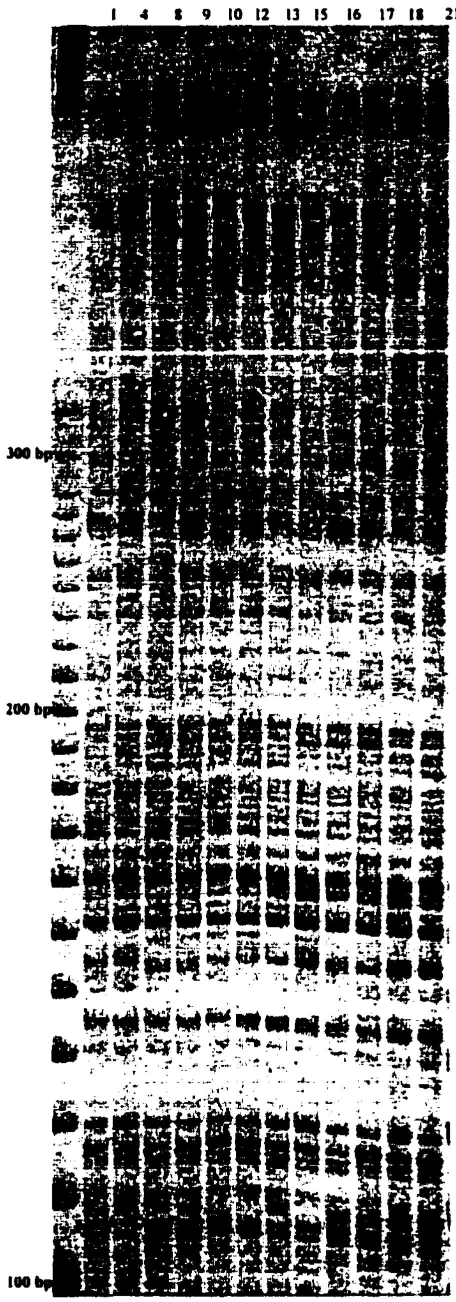
1 - Minnesota-Cambridge wild olive seeded
4 - Canada-Rosemount wild back seeded
8 - Oregon-Grand Island wild olive seeded
9 - Ontario, Canada-Huron County wild black seeded
10 - Nebraska-Panhandle Center wild tan seeded
12 - Wyoming-Platte County wild brown seeded
13 - Colorado-Weld County wild black seeded
15 - Colorado-Weld County wild tan seeded
16 - South Dakota domestic brown seeded
17 - Colorado domestic white seeded
18 - Colorado domestic orange seeded
21 - Colorado wild white seeded

Figure 4.2. AFLP pattern on twelve proso millet biotypes derived by the primer combination Mse-CTC/EcoR-TG. Numbers on gel correspond to biotypes listed on the right. DNA marker separated in 10bp is presented in the first column.



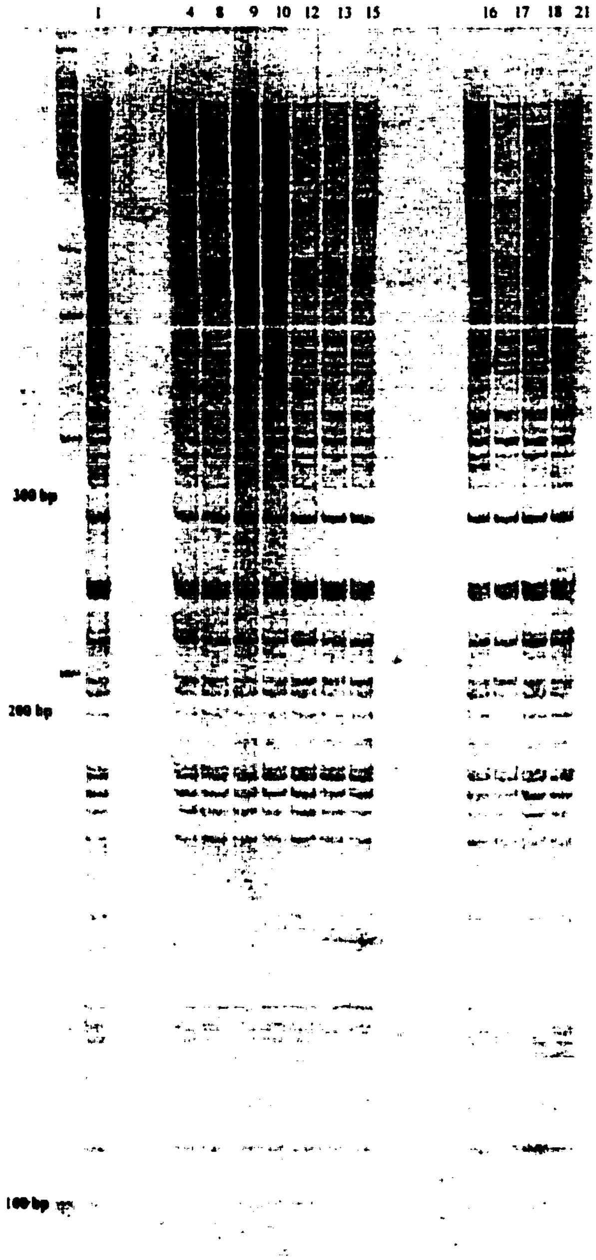
1 - Minnesota-Cambridge wild olive seeded
4 - Canada-Rosemount wild back seeded
8 - Oregon-Grand Island wild olive seeded
9 - Ontario, Canada-Huron County wild black seeded
10 - Nebraska-Panhandle Center wild tan seeded
12 - Wyoming-Platte County wild brown seeded
13 - Colorado-Weld County wild black seeded
15 - Colorado-Weld County wild tan seeded
16 - South Dakota domestic brown seeded
17 - Colorado domestic white seeded
18 - Colorado domestic orange seeded
21 - Colorado wild white seeded

Figure 4.3. AFLP pattern on twelve proso millet biotypes derived by the primer combination Mse-CAA/EcoR-AAG. Numbers on gel correspond to biotypes listed on the right. DNA marker separated in 10bp is presented in the first column.



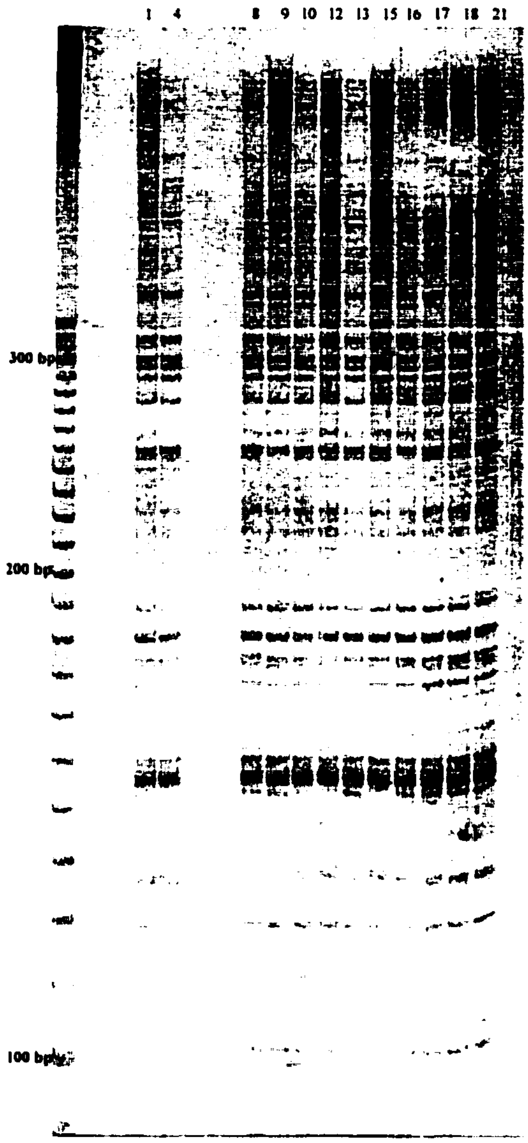
1 - Minnesota-Cambridge wild olive seeded
4 - Canada-Rosemount wild back seeded
8 - Oregon-Grand Island wild olive seeded
9 - Ontario, Canada-Huron County wild black seeded
10 - Nebraska-Panhandle Center wild tan seeded
12 - Wyoming-Platte County wild brown seeded
13 - Colorado-Weld County wild black seeded
15 - Colorado-Weld County wild tan seeded
16 - South Dakota domestic brown seeded
17 - Colorado domestic white seeded
18 - Colorado domestic orange seeded
21 - Colorado wild white seeded

Figure 4.4. AFLP pattern on twelve proso millet biotypes derived by the primer combination Mse-CTT/EcoR-AGG. Numbers on gel correspond to biotypes listed on the right. DNA marker separated in 10bp is presented in the first column.



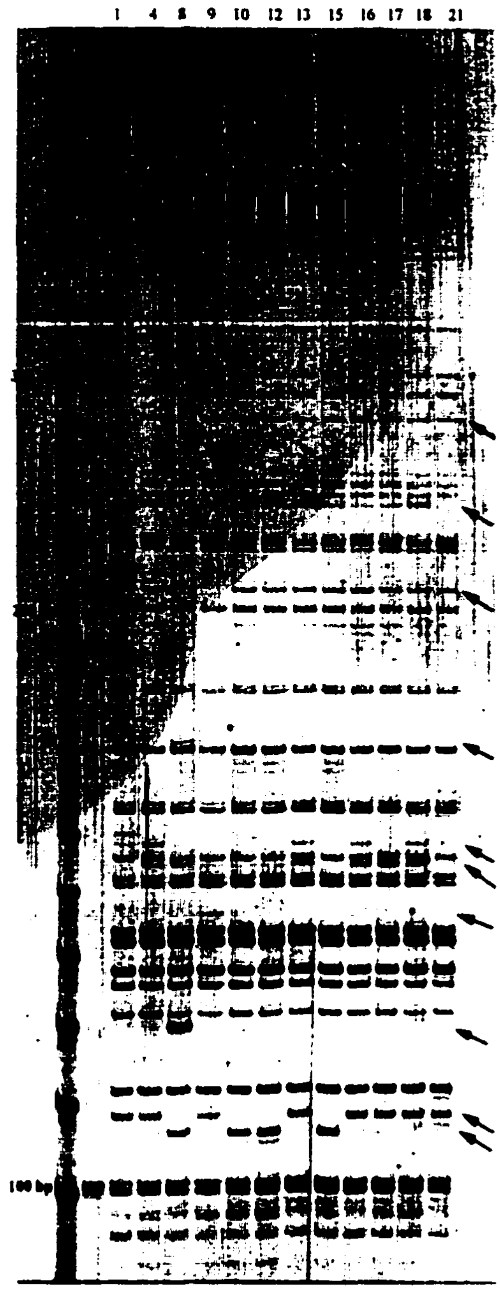
1 - Minnesota-Cambridge wild olive seeded
4 - Canada-Rosemount wild back seeded
8 - Oregon-Grand Island wild olive seeded
9 - Ontario, Canada-Huron County wild black seeded
10 - Nebraska-Panhandle Center wild tan seeded
12 - Wyoming-Platte County wild brown seeded
13 - Colorado-Weld County wild black seeded
15 - Colorado-Weld County wild tan seeded
16 - South Dakota domestic brown seeded
17 - Colorado domestic white seeded
18 - Colorado domestic orange seeded
21 - Colorado wild white seeded

Figure 4.5. AFLP pattern on twelve proso millet biotypes derived by the primer combination Mse-CAG/EcoR-AAG. Numbers on gel correspond to biotypes listed on the right. DNA marker separated in 10bp is presented in the first column.



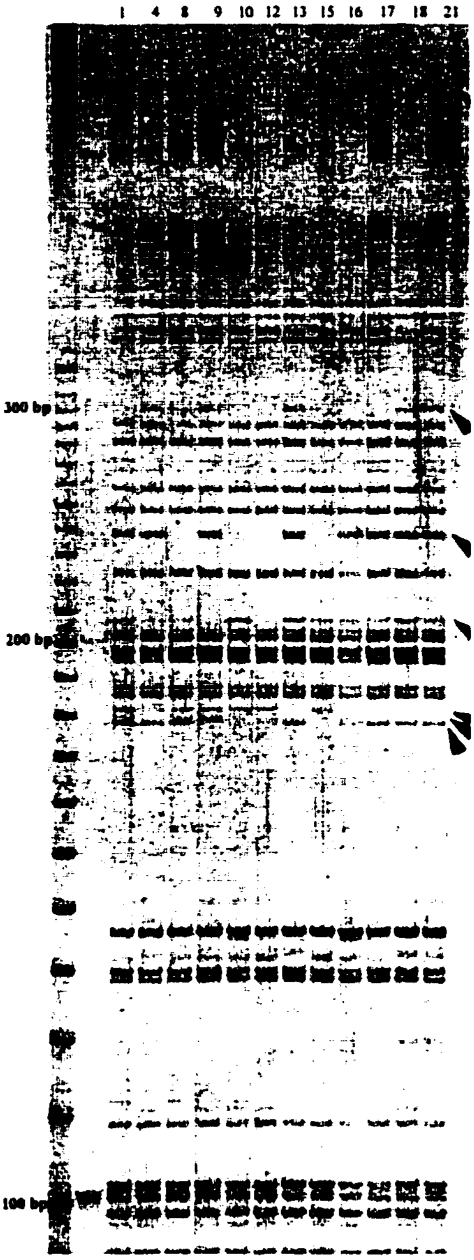
1 - Minnesota-Cambridge wild olive seeded
4 - Canada-Rosemount wild back seeded
8 - Oregon-Grand Island wild olive seeded
9 - Ontario, Canada-Huron County wild black seeded
10 - Nebraska-Panhandle Center wild tan seeded
12 - Wyoming-Platte County wild brown seeded
13 - Colorado-Weld County wild black seeded
15 - Colorado-Weld County wild tan seeded
16 - South Dakota domestic brown seeded
17 - Colorado domestic white seeded
18 - Colorado domestic orange seeded
21 - Colorado wild white seeded

Figure 4.6. AFLP pattern on twelve proso millet biotypes derived by the primer combination Mse-CTA/EcoR-AGG. Numbers on gel correspond to biotypes listed on the right. DNA marker separated in 10bp is presented in the first column.



1 - Minnesota-Cambridge wild olive seeded
4 - Canada-Rosemount wild back seeded
8 - Oregon-Grand Island wild olive seeded
9 - Ontario, Canada-Huron County wild black seeded
10 - Nebraska-Panhandle Center wild tan seeded
12 - Wyoming-Platte County wild brown seeded
13 - Colorado-Weld County wild black seeded
15 - Colorado-Weld County wild tan seeded
16 - South Dakota domestic brown seeded
17 - Colorado domestic white seeded
18 - Colorado domestic orange seeded
21 - Colorado wild white seeded

Figure 4.7. AFLP pattern on twelve proso millet biotypes derived by the primer combination Mse-CTT/EcoR-AAC. Numbers on gel correspond to biotypes listed on the right. DNA marker separated in 10bp is presented in the first column.



1 - Minnesota-Cambridge wild olive seeded
4 - Canada-Rosemount wild back seeded
8 - Oregon-Grand Island wild olive seeded
9 - Ontario, Canada-Huron County wild black seeded
10 - Nebraska-Panhandle Center wild tan seeded
12 - Wyoming-Platte County wild brown seeded
13 - Colorado-Weld County wild black seeded
15 - Colorado-Weld County wild tan seeded
16 - South Dakota domestic brown seeded
17 - Colorado domestic white seeded
18 - Colorado domestic orange seeded
21 - Colorado wild white seeded

Figure 4.8. AFLP pattern derived by the primer combination Mse-CAA/EcoR-ACTon twelve proso millet biotypes. . Numbers on gel correspond to biotypes listed on the right. DNA marker separated in 10bp is presented in the first column.

1 - Minnesota-Cambridge wild olive seeded
4 - Canada-Rosemount wild back seeded
8 - Oregon-Grand Island wild olive seeded
9 - Ontario, Canada-Huron County wild black seeded
10 - Nebraska-Panhandle Center wild tan seeded
12 - Wyoming-Platte County wild brown seeded
13 - Colorado-Weld County wild black seeded
15 - Colorado-Weld County wild tan seeded
16 - South Dakota domestic brown seeded
17 - Colorado domestic white seeded
18 - Colorado domestic orange seeded
21 - Colorado wild white seeded

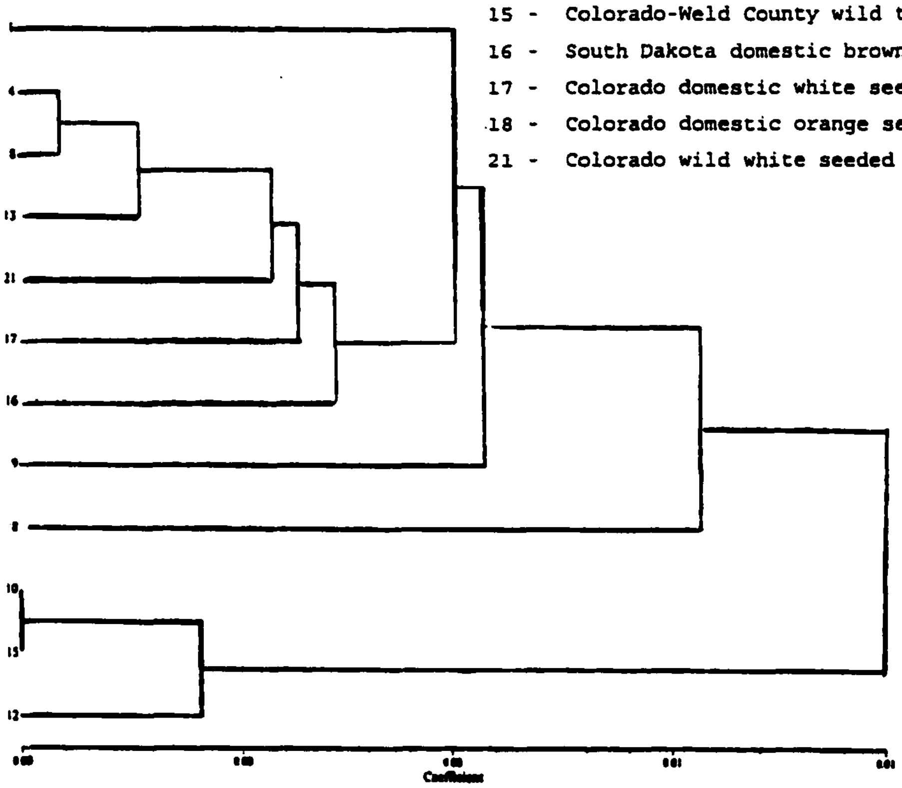


Figure 4.9. Dendogram of the twelve proso millet biotypes reveled by UPGMA cluster analysis based on NEI72 algorithmic. Numbers on the dendogram correspond to biotypes listed on the right..

1 - Minnesota-Cambridge wild olive seeded
4 - Canada-Rosemount wild back seeded
8 - Oregon-Grand Island wild olive seeded
9 - Ontario, Canada-Huron County wild black seeded
10 - Nebraska-Panhandle Center wild tan seeded
12 - Wyoming-Platte County wild brown seeded
13 - Colorado-Weld County wild black seeded
15 - Colorado-Weld County wild tan seeded
16 - South Dakota domestic brown seeded
17 - Colorado domestic white seeded
18 - Colorado domestic orange seeded
21 - Colorado wild white seeded

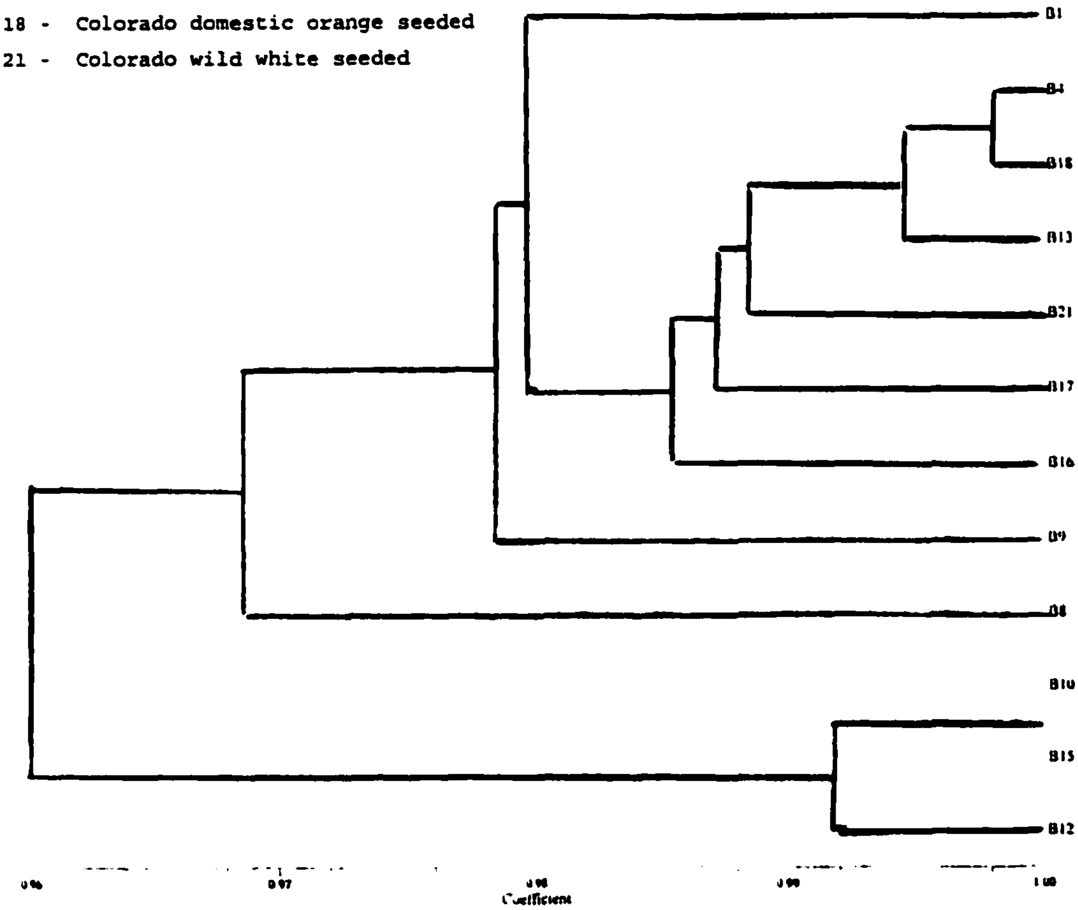


Figure 4.10. Dendogram of the twelve proso millet biotypes reveled by UPGMA cluster analysis based on DICE algorithmic. Numbers on the dendogram correspond to biotypes listed on the left.

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

GENERAL DISCUSSION

Growth analysis experiments (Chapter 2) show differences in the growth pattern among proso millet biotypes under noncompetitive conditions. These results confirm the observations reported by Eberlein et al. (1990), which indicated variation in growth and reproductive characteristics of proso millet biotypes. Seed color, weight and germination suggested differences among biotypes. Although differences were observed for seed coat as a percent of seed mass and germination rate, the results verified in this study differed from the results reported by Khan et al. (1996). Germination rate in their study was related to seed coat and seed color. In this study, that relation was not found. Black seeds had a different germination rate, even though no differences were observed in seed coat.

Plant growth analysis according to several authors (Dunan and Zimdahl 1991, Radosevich et al. 1997, Rejmanek et al. 1989) can be an indicator of competitive ability. Additive and replacement series experiments indicated different competitive ability between proso millet biotypes. These results depended on proso millet growth patterns and on crop growth characteristics. It was expected that crops with slower growth characteristics had greater reduction due to competition than crops with rapid growth. Plants emerging in an inappropriate environment where competing plants are more developed tend to suffer more competition than plants emerging in a non-competitive environment. The biotype that accumulated more shoot dry weight and had more leaf area was not the most competitive biotype. The fast growth observed for the Canada biotype was more important than increased leaf area or dry weight. This was shown by the shorter vegetative phase (24 days) for the Canada biotype compared to 37 days for the Colorado biotype. The results observed for nutrient uptake found differences in nutrient accumulation between proso millet biotypes. Rodriguez et al. (1990) found dissimilar result for domestic biotypes. Proso millet biotypes have the same resource requirements but the shortest life cycle determines the rate of needs. This may be the most important aspect of competitive relationships.

The AFLP procedure detected about 10% of polymorphic DNA bands among proso millet biotypes. Tsvelev (1984) reported that *Panicum* is a highly polymorphic genus that includes more than 500 species distributed in tropical and subtropical areas. Inter and intraspecific competitive ability is not well understood. Plant agressivity or competitive ability has been related to morphological and physiological characteristics (Cudney et al. 1989, Wall 1993, 1995, Tanji and Zimdahl 1997, Afentouli and Eleftherophorinos 1999). However no published reports were found that utilize genetic analysis associated with competition experiments to understand differences detected in competitive ability. This study did not determine if specific genes were responsible for differences in competitive ability but it will be an initial tool for future research combining the two important aspects in plant science: (i) biology and (ii) molecular genetics.

Two other issues are important for this discussion: (i) the importance of biology, ecology and genetic interaction to compare competitive ability of biotypes and (ii) the order in which experiments are conducted. This dissertation shows the importance of the three methods to analyze competitive ability. The use of only one method might result in wrong conclusions as seen in the biology experiments. Differences in competitive ability were explained by the results observed in the growth analysis and germination experiments. Genetic studies should be conducted first to detect the most distinct biotypes. Subsequently, biology and ecology experiments should be done. This order change would simplify subsequent studies.

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