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GENETIC VARIATION IN THE PHOTOSYNTHETIC ACTIVITY OF ASPARAGUS (*Asparagus officinalis* L.) CULTIVARS AND LINES

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GENETIC VARIATION IN THE PHOTOSYNTHETIC ACTIVITY OF ASPARAGUS (*Asparagus officinalis* L.) CULTIVARS AND LINES

By

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ABSTRACT

GENETIC VARIATION IN THE PHOTOSYNTHETIC ACTIVITY OF ASPARAGUS (*Asparagus officinalis* L.) CULTIVARS AND LINES

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Photosynthetic rates of eight asparagus genotypes were measured in the field from July to September, 1995, using an open system measuring whole plant photosynthesis. Patterns of daily changes and seasonal changes of asparagus plants' photosynthesis were studied and established. Significant differences ($Pr<0.1$) in photosynthetic rates among eight genotypes were found. Correlation between photosynthetic rate and yield was found to be significant ($Pr<0.05$), high yielding genotypes tending to have high photosynthetic rates. Differences in specific leaf weight (SLW) of the eight asparagus genotypes were significant ($Pr<0.05$) and its correlation with photosynthetic rate is significant ($Pr<0.05$). Genotypes with high SLW had high photosynthetic rate. Chlorophyll contents of the eight asparagus genotypes were measured, and highly significant differences in chlorophyll content were found ($Pr<0.01$). Rubisco activities of three asparagus genotypes were studied. Significant differences in rubisco activities exist among these three genotypes.

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INTRODUCTION

Asparagus (*Asparagus officinalis* L.) is an important perennial vegetable crop in Michigan. Yield trials of asparagus cultivars and lines have been conducted at two locations in Michigan since 1988. A wide variation of yield performance has been observed. Literature on the study of the relationship between photosynthetic ability and yield has presented two different opinions; one is that there is no direct relationship between photosynthetic rate and yield (Elmore, 1980); a contrary opinion shows that photosynthetic rate is positively correlated with yield (Zelitch, 1982). This experiment studied photosynthetic rate of eight asparagus genotypes in an attempt to determine the relationship between photosynthetic rate and yield in asparagus. Studying photosynthesis of the whole plant under field conditons is more likely to reveal the true aspects of this critical physiological activity, yet none of the previous studies on asparagus photosynthesis were conducted under field conditions and measuring photosynthetic rate on a whole plant basis, nor did any of the previous studies on asparagus photosynthesis deal with the variability of photosynthetic rates among different genotypes. This experiment was carried out in the field measuring photosynthetic rates of whole asparagus plants. This has several advantages over indoor photosynthetic rate measurements on plant parts. Studies on the patterns of daily and seasonal changes of asparagus photosynthetic activity were made possible by these field measurements.

Ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco) is the most abundant leaf protein in green plants and the key enzyme in CO_2 fixation. It catalyzes the following reaction in plants:

$$\text{Ribulose-1,5-bisphosphate} + CO_2 + H_2O \xrightarrow{Mg^{++}} 2 \text{ x 3-phosphoglycerate} + 2H^+$$

This is the first step of CO_2 fixation in C_3 plants. To test if there are significant differences in rubisco activity among different asparagus genotypes, and to see if high rubisco activity is associated with high photosynthetic rate, I studied the rubisco activities of three genotypes having large differences in photosynthetic rates.

The hypotheses tested in this experiment were: (i) that there are significant differences in photosynthetic ability among different asparagus genotypes; (ii) that high photosynthetic rate is positively correlated with high yield performance; and (iii) that high rubisco activity is associated with high photosynthetic rate.

Other goals included: (i) establishment of the patterns of daily and seasonal photosynthetic rate changes of asparagus plants; (ii) study of factors limiting photosynthetic rate and plant traits associated with photosynthetic rate; and (iii) study of the chlorophyll and protein content of asparagus fern, and the variability of chlorophyll content among eight asparagus genotypes.

Chapter One: Literature Review

Part One: Basic information on asparagus.

Asparagus officinalis L. is a very unique vegetable crop, popular in many parts of the world. A definition given in the "Hortus Third" (L. H. Bailey Hortorium, 1976) describes it as a "hardy perennial grown for its edible spring shoots, which are tender and can be injured by frost ". Taxonomically, asparagus is a member of the family *Liliaceae* and belongs to the genus *Asparagus* in which it is the only species cultivated for food use.

Asparagus is native to the eastern Mediterranean area. (Luzny,1979; Tutin et al., 1980). It is grown commercially in temperate and tropical climates throughout the world. Asparagus was among the first crops brought to the new world and during the 1800s it became widely distributed in many states. (Gleason and Cronquist, 1963). Michigan has been a major producer of asparagus in the United States, and as such it is one of the most important horticultural crops in Michigan.

Asparagus has several characteristics which distinguish it from many other vegetable crops. It is a perennial vegetable crop, and its economic harvest life can last as long as 20 years. (Pierce, 1987).

Another unique character is its photosynthetic organ. Its leaves are reduced to scale-like bracts. Leaf function, photosynthesis, is performed by special twigs called cladophylls which posses the necessary photosynthetic components to function as leaves. The cladophylls usually are borne in fascicles of three to eight in the axils of the leaf scale.(L. H. Bailey Hortorium, 1976). Because of this, asparagus has a rather special photosynthetic apparatus. The shading effect within the plant is small, and this can be a

big advantage in photosynthesis compared to other leaf types; and ambient CO_2 is more easily accessible to photosynthetic cells from all directions around the cladophyll.

Asparagus is a dioecious species with male and female plants existing as separate individuals.(L. H. Bailey Hortorium, 1976). Hence there are male flowers (stamens only) and female flowers (pistils only) in asparagus on separate plants. The male flowers are slender, bell-shaped and greenish white in color. Each flower has an aborted ovary and a well developed anther bearing pollen. Female flowers are smaller than male flowers and contain vestigial, functionless anthers and a well developed ovary, style and three feather-like stigmas. (Flory, 1932). Occasionally perfect flowers can be found in male plants; these are called andromonoecious plants. These flowers have the ability to produce seeds like female flowers. The pistillate and perfect flowers produce small, round berries containing a few seeds. The phenomenon permitting andromonoecious plants to produce seeds is important in asparagus breeding. Seeds collected from perfect flowers will give 3 male plants to 1 female instead of the approximately equal numbers produced by seed from female plants. Of the 3 males, two will be ordinary males with the genotype Mm, but the third has a " double dose " of maleness with a genotype MM. They look alike but can be distinguished from one another by making test crosses. Then if the supermale plant is used as a male parent, the resulting progeny will be all male. By this means it is possible to produce an all-male population. (Rick and Hanna, 1943). This effort is undertaken because male plants have several advantages over female plants in production; male plants develop earlier and produce a larger number of shoots than female plants, and they live longer than female plants; therefore, giving a greater total weight of crop. Comparing the underground portion, staminate crowns are larger than pistillate crowns and they have more buds than female crowns. Also male plants do not produce seedling asparagus plants, which are considered weeds, because they compete with the established crowns and may favor disease epidemics. (Sneep, 1953). Another advantage of all-male hybrids is that growers are unable to produce seed and thereby alter

the original cultivar. This practice led to the loss of excellent original strains of 'Martha Washington' and 'Mary Washington'. (Ellison et al.,1985). In fall there is no significant difference between the weights of all above-ground parts of male and female plants, but in female plants about a third of the above-ground weight is the effect of berries, which have no significance for carbon assimilation. (Robbins et al., 1925). For these reasons, breeding efforts are concentrated on developing all-male cultivars. All-male cultivars such as 'Jersey Giant' are being used widely in commercial production, and it can be predicted that they will be dominant in future asparagus production.

A characteristic of growth of the asparagus plant is an underground organ called a crown, from which both stems and roots grow. Crowns consist of many unbranched fleshy storage roots up to 6mm thick attached to the closely spaced basal internodes of an underground stem (Blasheng, 1932). Below the crown, there are two types of roots, storage roots, which are thick and fleshy and serve as plant food reserves, and fibrous roots, which serve to absorb water and nutrients from the soil. The fibrous roots die after each year's growth, the fleshy roots die after providing nourishment for the next generation of spears. (Shelton et al., 1978). Young shoots, composed of young stems and terminal buds, are produced from the crown annually in the growing season.

Asparagus is predominantly grown in temperate climates. When grown in such climatic areas, the fern senesces in the fall and the plant remains dormant during winter. (Morse, 1916; Carolus, 1962). In the spring, buds on the underground stem grow to produce new shoots.(Tiedjens, 1926; Kretschmer and Hartmann, 1979). The main period of bud formation occurs in the previous growing season, during the fern growth period following harvest of spears. (Tiedjens, 1924,1926). The time required for a new spear to develop is related to the growth of other spears on the rhizome. As a spear grows beyond marketable size, growth of the adjacent bud is suppressed more strongly (Tiedjens, 1926). The edible portion of the plant is the tender, young, unexpanded spears which usually are harvested from mid-spring to early summer, after which the fern is allowed to develop

fully. Between the formation of full fern and the fall frost, asparagus photosynthesizes actively and stores carbohydrate in fleshy roots. After the fall frost occurs, the asparagus plant goes into a resting period until the next year's spear production.

Asparagus has a wide geographic distribution and adapts to very diverse environmental conditions; it can live and in some measure thrive on almost any kind of soil.

In commercial production asparagus fields are established by planting seedlings. It has been confirmed by a number of studies that one-year-old asparagus crowns are most suitable for use in planting. Customarily, the first asparagus harvest begins in the third season after crown planting; harvesting reaches its peak in the 7th or 8th harvest season. (Dufault et al., 1983).

Fern vigor of asparagus had been thought to be correlated with its yield. In a study on the relationship between yield and brush vigor in asparagus (Ellison et al.,1959) brush vigor was measured in two aspects: (1) number of stalks per plant, and (2) an index based on relative size of stalks per plant. The index was computed by rating small stalks (diameter less than 10mm) as 1, medium stalks (10-15mm) as 2, large stalks (16 mm and greater) as 3, then total fern index (TFI)=3 x (no. of large stalks) + 2 x (no. of medium stalks) +1 x (no. of small stalks). It was found that yielding ability of individual male and female asparagus plants is significantly correlated with fern vigor. Positive correlations also exist between number of spears and number of stalks and spear diameter and stalk diameter. (Ellison et al., 1959).

In asparagus, as in many other crops, there has been a transition in production from using open-pollinated cultivars to planting more and more hybrids. In two reports (Benson et al., 1980, 1982) it was found that yield, vigor, and biomass accumulation of new hybrid cultivars are far superior to open-pollinated cultivars. The F_1 crowns produced greater weight and more fleshy roots earlier in the growing season. It also was found that the greatest difference in growth between an OP and F_1 cultivars was greater

partitioning of dry matter in the F_1 root systems early in the growth of the seedling. The considerably greater partitioning of dry matter into the root system of F_1 early in the growth of the seedling is quite significant. An enlarged root system early in development should allow an asparagus plant to produce greater fern biomass, because of its greater absorptive area, and ability to store greater amounts of carbohydrates for future growth.

Various studies indicated that asparagus is drought-tolerant and salt-tolerant. (Welcox-Lee, 1987). In fact, salt may be used to control weeds in commercial production. Salt and drought tolerance can be explained by the very deep and widespread root system of asparagus. Asparagus roots can be as long as 10 feet (Kidner, 1959).

In Taiwan, a very special asparagus production system was developed which utilizes the longer growing season of the tropical climate there, and it is called the "mother stalk-cultivation method". The winter temperatures in Taiwan remain above 16°C. Because of this high temperature, the fern does not die off in the fall and can remain photosynthetically active during winter. The harvest is divided into two periods: a spring harvest from April to July; and a fall harvest from August to November. (Lin, 1979). During harvest, mother ferns (2 or 3 per plant) are allowed to develop and remain, whereas subsequent spears are harvested, thus maintaining a balance between carbohydrate production and carbohydrate removal. (Robb, 1984).

The biggest problems in asparagus production are two fungal diseases: *Fusurium oxysporum* f.sp. *asparagi* (FOA) and *Fusurium moniliforme* (FM). FOA is the causal agent of the wilt and root rot disease and FM is the casual agent of the stem and crown rot disease. These two disease are the limiting factors for asparagus production in the United States and they have been responsible for the decline of asparagus production in Michigan. Great efforts have been made to breed cultivars which are resistant or tolerant to these diseases. (Elison, 1994).

Part Two: Previous studies on the photosynthetic activity of asparagus

Photosynthesis is a critical physiological activity of all plants. For asparagus, the spears that come out of the ground in the spring draw their structural materials from roots, where food elaborated by the ferns in the preceding year is reserved. Thus, study of photosynthesis of asparagus is very important to understanding this process of assimilation and for any possible improvements in this area.

The earliest work on asparagus photosynthesis might be the report from Sawada et al.(1962). In this report they gave a general description of the structure of cladophylls, daily change of assimilation, assimilation rates of male and female plants, and the influence of temperature on assimilation rate. In asparagus, photosynthesis is carried out by cladophylls. In the central part of a cross section of a cladophyll there are several vascular bundles, surrounded by a tissue which looks like palisade tissue of broad-leaf plants; the outermost part of it is covered with epidermis. Many stomata are scattered over the entire surface of the cladophyll. Having such a unique structure, the cladophyll may be considered as a "cylindrical leaf." Daily changes of assimilation were studied by taking samples of needles at intervals of several hours in a day and determining their assimilate content. Analysis revealed that the assimilate content increased steadily as the time of a day advanced, until about 3:30 PM, after of which it began to fall. It was concluded that the peak of assimilate content occurs at about 3:00-4:00 each day. Assimilation rate was determined by taking the difference between assimilate content measured at different times in a day; the range of assimilation rate varied from 91 to 167 mg glucose per 5 g of fresh needles. Assimilation was affected directly by the prevalent weather conditions; there exists a close parallel between daily assimilation and solar energy. It was stated within the scope of their experiment that no definite difference was found between male and female plants in their assimilation ability. To study the relationship between temperature and assimilation rate, the plants were put into three different rooms in a phytotron with temperatures held at 13°C, 18°C and 28°C,

respectively. It was found that with the temperatures employed, assimilation was most efficient at 18°C. The methods and units used in this experiment were very different from those ordinarily used in photosynthesis research.

In subtropical Taiwan, asparagus became one of its most important cash crops, and Taiwan became the world's leading exporter of asparagus. Because of this importance, much asparagus research has been carried out in Taiwan, and two Taiwan papers related to asparagus photosynthesis research are discussed below.

In the paper "The Photosynthesis of Asparagus Plant" (Lin et al.,1978), photosynthetic activity of asparagus was studied at six different stages of development, from spear formation to yellowing cladophylls. The photosynthetic rate was measured by placing spears or small branches of asparagus into an assimilation tube; net assimilation rate was obtained based on the difference of CO_2 concentration in and out of the tube. It was found that at spear stages, from the beginning of spear growth to spear branching , there was no net photosynthesis, but about 50-60% of the CO_2 released by dark respiration can by refixed by spears. This result is consistent with another study on asparagus photosynthesis.(Downton et al., 1975). When young cladophylls are developed, about one month after the growth of a spear, the net photosynthetic rate can be as high as 48.9 mg CO_2 /g f.wt./ h. Dark respiration rate is highest at this stage (23 mg CO_2/g f.wt./h). The photosynthetic rate reaches a peak when the plant reaches the vigorous cladophyll stage, about two months after spear emergence; the net assimilation rate can be 65.3 mg CO_2/g f.wt./h as measured. Photosynthetic rate drops to 8.6 mg CO_2/g.f.wt/hr when cladophylls are yellow. They also studied the photosynthetic rate of male and female plants and found that there is no significant difference between them; their observation confirmed the conclusion reached by a previous study. (Sawada et al., 1962).

In another study from Taiwan (Lin, 1983) on asparagus photosynthesis, the photosynthetic rate was measured by cutting two to three asparagus twigs and sealing

them into an assimilation tube. An infrared CO_2 gas analyzer was used to measure differences of CO_2 concentration. Photosynthesis was measured at temperatures of 15°C, 20°C, 25°C, 30°C, and 35°C, and it was found that the optimum temperature for photosynthesis was about 20°C. Photosynthetic rate also was measured at two levels of O_2 concentration, 2% and 21%. The increase of photosynthetic rate ranges from 29% to 66% in 2% O_2 compared to the 21% O_2 level under the six light intensities employed. The photorespiration rates observed were 1.07-4.6 mg CO_2/g f.wt./h. The ratio of photosynthesis to respiration in the fern is 2-5:1. The respiratory quotient (Q_{10}) is about 2. From the fact that photorespiration is high in the fern, and photosynthesis is enhanced at low oxygen concentration, the author concluded that asparagus must be a C_3 plant. The photosynthetic rate of asparagus was shown to be high in the above studies; the author speculated that this might be because asparagus has a plant form, different from other crops. The photosynthetic organs, the cladophylls, have the shape of needles and thus the shading effect of leaves is very slight. Light can penetrate easily into the inner part of the asparagus plant , so that the whole plant can engage fully in photosynthesis.

The percentage of F_1 hybrid cultivars of asparagus is increasing in commercial production over open-pollinated cultivars (OP). A study was carried out by Benson et al.(1980) in California to investigate the partitioning of dry matter in these two kinds of cultivars. It was found in this study that the F_1 plants, with about the same photosynthetic area, produced more total dry weight than the OP plants. This suggests that the photosynthetic efficiency of hybrids is higher than OP cultivars. Combining this with the evidence from Dirks et al.(1982) I propose the hypothesis that yield differences among varieties may result from differences in their photosynthetic efficiency and/or distribution of assimilates.

A more thorough study of asparagus photosynthetic activity was conducted by Downton et al.(1975) in Australia. In this work, photosynthetic activity was assayed at four stages of asparagus growth: 1) spear, in commercial form. 2) spears with head

losing compact structure, 3) pre-fern stage resulting from extensive elongation of main stem and side branches, and 4) fern with fully developed cladophylls. The photosynthetic pathway of asparagus was elucidated by a $^{14}CO_2$ incorporation experiment; for this purpose PEP carboxylase and RuBP carboxylase activity was measured. CO_2 exchange rate was measured by placing detached shoots with bases in water and using an infrared CO_2 analyzer. Results showed that at two spear stages there was no net uptake of CO_2, even at the 2% O2 level. Nonetheless, spears can reassimilate 50-100% of the carbon dioxide produced in respiration. Net photosynthesis became measurable once the spears began to assume a fern-like form (stage 3). The photosynthetic rate at this pre-fern stage is about 17 μmol CO_2 h^{-1} mg^{-1} chlorophyll but the dark respiration rate is very high at this stage, with a value of 26.3 μmol CO_2 h^{-1} mg^{-1} chlorophyll. The net photosynthesis in fully differentiated fern exceeded dark respiration with values 20.9 μmol CO_2 h^{-1} mg^{-1} chlorophyllfor net photosynthetic rate and 11.7 μmol CO_2 h^{-1} mg^{-1} chlorophyllfor dark respiration. There was a 30% enhancement of photosynthesis at low oxygen concentration (2%), which is typical of C_3 plants. The chlorophyll content of asparagus at the fern stage was calculated to be 1.35 mg g^{-1} fresh weight. The RuDP carboxylase at the fern stage was 10.7 μmol CO_2 min^{-1} mg^{-1} chlorophyll; this seems to be one of the highest measured in a C_3 plant. On the other hand, PEP carboxylase activity was low in the fern cladophylls, as low as 0.71 μmol CO_2 min^{-1} mg^{-1} chlorophyll, indicative of the C_3 pathway for asparagus photosynthetic activity. Another character related to photosynthetic ability, stomatal frequency, was studied in this work; asparagus cladophylls have a high density of stomatal frequency, which is 127.5 stomata/mm^2. The cladophylls, being thin, cylindrical, leaf-like structure with a high density of stomata seem well adapted for CO_2 exchange with the atmosphere.

Photosynthesis of asparagus under different water stress was studied by Drost et al.(1990). Asparagus (cv. Jersey Giant) seedlings were grown in the greenhouse under three levels of water stress, -0.05, -0.3, and -0.5 MPa. Diurnal change in net assimilation

rate (A) and stomatal conductance (gs) were measured under these conditions. Measurements were midway up the fern on young fully expanded second order branches. Net CO_2 assimilation rate was measured with an ADC model LCA-2 infra-red CO_2 gas analyzer and a Parkinson broadleaf chamber. Decreasing soil matrix potentials decreased all measured parameters, but did not alter the diurnal pattern. A and gs increased to their maximum at 10 AM and then decreased as the day progressed. This pattern is different from the results obtained by Sawada et al.(1962). Water stress caused reductions both in A and gs, and reductions in A were correlated closely with g, indicating that water deficits reduce photosynthesis through stomatal closure.

The above studies on asparagus photosynthetic activity were performed with a small portion of the whole plant, and measurements were carried out under indoor conditions for a short period of time. No research has been conducted under field conditions measuring whole asparagus plant gas exchange during a whole growing season. There also has been no work on the variation of photosynthetic ability among currently utilized asparagus cultivars.

Part Three: Previous studies on variation of photosynthetic ability among different genotypes of various crops and studies on the relationship between photosynthetic rates and yield.

Varietal differences in net photosynthetic ability are well documented in major agronomic crops like wheat, maize, oats, soybean and sugarbean, and also in a few important vegetable crops including tomato and sweet pepper.

Among the better crop examples involving this kind of study is tomato, as evidenced by the titles of following papers: "Genotypic Variation in Carboxylation of Tomatoes" (Augustine and Stevens, 1976); "Physiological, Morphological, and Anatomical Studies of Tomato Genotypes Varying in Carboxylation Efficiency" (Augustine and Stevens, 1979); "Difference Between Tomato Genotypes in Net Photosynthesis and Dark Respiration" (Van de Dijk and Maris, 1985); "The Influence of Irradiance and External CO_2-Concentration on Photosynthesis of Different Tomato Genotypes" (Nilwik et al., 1982); "The Influence of Temperature on Photosynthesis of Different Tomato Genotypes" (Gosiewski et al., 1982).

Results from the experiment of Augustine et al., (1979) showed that specific leaf dry weight (SLDW) and leaf thickness was greater in genotypes with high carboxylation efficiency (CE). It was indicated that differences in gaseous diffusion potential may account in part for genotypic differences in CE. Differences in photosynthetic rates may also result from the adaptation of different cultivars to growth conditions. As in this experiment, five cultivars were developed at different locations, and some of them may have suffered from lack of adaptation to local conditions. In another experiment by Augustine et al. (1976) they studied the gas exchange characteristics of 24 genotypes of tomato varying in growth habit, leaf morphology, chlorophyll content, and rubisco activity. Significant genotypic differences for carboxylation efficiency were observed.

Photosynthesis of the whole tomato plant was measured by Gosiewski et al. (1982), involving 10 genotypes. Positive correlations were found between net photosynthesis, net CO_2 uptake on a daily basis, specific leaf weight, and leaf area ratio. Another study (Nieuwhof et al., 1988), reported that differences in net photosynthesis among genotypes of tomato can be attributed mainly to differences in the capacity of the leaves to absorb light as a result of variation in chlorophyll content and leaf thickness. Considering the above, we can see differences in net photosynthesis among tomato genotypes quite often are related to parameters such as specific leaf dry weight (SLDW), leaf area ratio (LAR), leaf thickness, gaseous diffusion potential, chlorophyll content, light absorption, adaptation to growth conditions, and rubisco activity.

A Study of net photosynthesis of soybean leaves by Dornhoff and Shibles (1970) showed that 20 soybean varieties differed significantly in net photosynthesis. Differences of stomatal resistance and mesophyll resistance were found among genotypes. Evidence suggests that varietal differences in net photosynthesis were caused by differences in diffusive resistance. This result was in agreement with one explanation for differences of net photosynthesis among tomato genotypes. Among the 20 varieties used in this experiment, there is a trend toward high photosynthetic rates in high-yielding varieties, but there were some exceptions to these trends. In another study on soybean photosynthesis by Curtis et al. (1969), photosynthetic rates in seedlings of 36 soybean varieties were measured to determine whether significant varietal differences exist. A large variation was found among these varieties. However, large yield differences frequently were obtained with the same variety at different locations.

Photorespiration rates of different genotypes of both tomato and soybean were measured; no significant variations were found in photorespiration among different genotypes, and varietal differences in net photosynthesis were not associated with differential photorespiration rates.

Gaastra(1962) stated that photosynthesis is composed of three main processes: (1) a photochemical process involving the utilization of light energy, (2) a diffusion process for transporting CO_2 to the fixation site, and (3) biochemical processes involving the fixation and chemical reduction of CO_2. Variation in photosynthetic rates within a species may result from variability in one of these three aspects, or a combination of variations.

In the review article "The Paradox of No Correlation Between Leaf Photosynthetic Rates and Crop Yields" (Elmore, 1980) the author pointed out that photosynthetic rates have been shown to differ within species on numerous occasions. The author listed reports of such difference in species such as *Triticum aestivum* L., *Zea mays* L., *Glycine max* L., *Oryza sativa* L.,*Oryza indica* L., *Gossypium hirsutum* L., *G. arborum* L., *Medicgo sativa* L., *Dactylis glomerata* L., *Avena sativa* L., *Coleus spp.* , *Lycopersicon esculentum* L., *Festuca arundinaceae* L., *Phaseolus vulgaris* L., *Hordeum vulgare* L., *Pyrus malus* L., *Prunus persica* L. Yet, the author stated that there was little experimental evidence of any positive relationship between yield and leaf photosynthetic rate, or that there is any instance in which selection for a greater rate of leaf photosynthetic has led to increased yield.

The same opinion was shared by Evans (1975), who claimed in an article that there is little evidence of any positive relationship between yield and the rate of CO_2 exchange per unit of leaf area.

This opinion was challenged in another review article entitled "The Close Relationship Between Net Photosynthesis and Crop Yield" (Zelitch, 1982). The author disagrees with the prevalent opinion that no relationship exists between rate of CO_2 exchange and crop yield. He pointed out that key problems such as instantaneous measurements conducted under standardized conditions, rather than seasonal measurements conducted in the field led to such conclusions. It was stated further that in all such papers photosynthesis was measured instantaneously (short-time), often on a

single occasion, and usually on a specific leaf position at a single stage of development, in bright light at constant temperature, and frequently under ideal lab conditions. Such assays may have no relation to net seasonal assimilation of CO_2 by the entire plant and its translocation to the harvested plant organ, upon which yield is ultimately based. The shortcoming of measuring leaf photosynthesis is that individual leaves in a community will vary greatly in their net CO_2 assimilation rate, depending on the irradiation reaching that leaf, temperature, water, other climatic factors, and stage of development. Photosynthetic rates measured under different experimental conditions may reflect a plant's adaptation to such conditions, and differences in photosynthesis associated with them may rarely express themselves in the field where such conditions don't prevail.

In view of the above, it is no wonder that photosynthesis has rarely been associated with yield. So to the contrary, the author believes that crop yield is closely related to the net photosynthetic assimilation of CO_2 throughout an entire season. Increasing the rates of net photosynthesis and enlarging the storage capacity by selection and breeding may bring about large increases in yield, especially in C_3 species. From the fact that about 90% of the dry weight of higher plants is derived from CO_2 assimilation it seems obvious that higher rates of photosynthesis should lead to higher yields.

Some evidence was given by Zelitch to support his opinion. Christy and Porter (1982) worked on soybean CO_2 assimilation in the field with portable chambers and evaluated CO_2 exchange in replicate plots continuously during daylight hours for an entire season. The cumulative net photosynthesis for two varieties for two seasons was compared with their respective yields. The correlation was nearly perfect, $r=0.98$. Puckride (1971) presented carbon budgets of two wheat varieties grown in three successive years; the average grain yields (g $(m^2)^{-1}$) were 651, 309, and 315 for the three years, and the relative net CO_2 assimilation after anthesis by the canopy were 100, 41, and 31 respectively, showing that grain yield was closely related to seasonal carbon uptake.

The measurements of net photosynthesis on individual leaves and whole plants of tobacco were compared. (Peterson and Zelitch, 1982). It was found that on any given day there was considerable variation in CO_2 uptake per unit leaf area at different leaf positions. Over the course of the season there was a poor relationship between CO_2 uptake per unit area and individual leaf positions. Clearly, a limited number of instantaneous measurements can lead to large errors when estimating photosynthetic assimilation under field conditions. However, measurements of total CO_2 uptake per plant were in good agreement with actual dry weight increases over the course of the season.

Examples also exist of the success of breeding higher yielding cultivars as a result of selecting on the basis of photosynthetic rates. A comparison of photosynthesis and yield among wild species and modern peanut cultivars selected for higher yield made by Bhagsari and Brown (1976a) is a good example. The cultivated plants had a mean net photosynthesis of 23 mg CO_2 $(dm^2)^{-1}$ h^{-1}, whereas wild species had a mean of 19 mg CO_2 $(dm^2)^{-1}$ h^{-1}. The peanut cultivars developed for high yield had high photosynthetic rates, indicating that an indirect selection for high photosynthetic capacity may have been made inadvertently. There are other reports that high-yielding hybrid cultivars have higher photosynthetic rates than their parents, such as in maize as reported by Vietor and Musgrave (1979).

Photosynthetic rate is only one factor in dry matter accumulation and yield formation. Yield is a very complex trait controlled by many factors such as dark respiration, nitrogen metabolism, translocation, and partitioning of photosynthate. It seems that none of these plays a dominant role in the process, but that they work collectively to determine yield. Thus, selection for high photosynthetic rate may lead to increase in yield, only if it is not at the expense of other factors such as whole leaf area, translocation, storage capacity, etc.

What are the possible means to improve photosynthetic rates of a crop? We need to look at elements involved in this process. It seems variation in chlorophyll content generally has little impact on variation in CO_2 exchange rate (CER), because even chlorophyll-deficient barley mutants have near-normal CER. (Ferguson et al., 1973). In the dark reaction, variation in ribulose bisphosphate carboxylase (rubisco) in C_3 species has been found to correlate with leaf CER. (Randall et al., 1977). Selecting for high rubisco activity might be at the expense of other important enzymes, as it represents about 50% of the soluble protein of leaves. Selection for high specific activity forms of rubisco might be better. A morphological character, specific leaf weight (SLW=leaf dry weight per unit area), often is found to correlate with CER. (Barnes et al., 1969; Dornhoff et al., 1970). This is suggested in the above papers as a criterion in selection for high photosynthetic rate plants. Considering the complexity of the controls in photosynthetic rate and the lack of success at improving it by selection for component processes, selecting photosynthetic rate directly might be more effective. There was much evidence of heritable variation in CO_2 exchange rate, and high and low lines have been selected in several species. (Crosbie et al., 1978; Vietor et al., 1979; Wilson et al., 1969).

Improvements in crop yield have been realized largely by increasing harvest index, which is the ratio of economic sink to total plant weight, but clearly there is a limit to how far this can go. Improvement in the maximum rate of leaf photosynthesis may then become essential to further increases in yield. This could be realized, because CO_2 and light enrichment studies have indicated that crop yield frequently is limited photosynthetically. (Gifford and Evans, 1981).

Chapter Two: Materials and Methods

Part one: Field study.

A: Plant materials.

A field asparagus photosynthesis study was carried out during the summer of 1995 at two locations in Michigan. Plant materials used in this experiment were from two existing replicated cultivar trials. Yield trials of asparagus cultivars were established in 1988 at two locations in Michigan, Southwest Michigan Research and Extension Center (SWMREC) near Benton Harbor, Mich. and Max Kokx Farm near Hart, Mich. One-year-old crowns were planted in April, 1988 at the two locations. At SWMREC 36 hybrids and open-pollinated cultivars were used; at Hart 34 of the same 36 cultivars were employed. Five of the eight entries in this asparagus photosynthesis study were taken from this yield trial. Considerations in selecting cultivars were wide variations in several aspects such as yield performance, country or area of cultivar development, cultivar type, and plant architecture. The selections were '44Px22-8', 'Jersey Giant', 'Franklim', 'UC86-11', and 'Tainan No. 3'.

'Jersey Giant' is a successful all-male asparagus hybrid. Its pollen parent is N.J. supermale 22-8, the 8th seedling of the S_1 generation of male plant No. 22-8 (Ellison, 1985). Supermale No. 22-8 is also the pollen parent of 44Px22-8, another cultivar used in this experiment. It is homogametic for the male gene (MM), and produces all-male offspring (Mm) when crossed with a female (mm). 'Franklim' is a superior all-male hybrid developed in Netherlands; it has performed well in this yield comparison experiment. 'UC86-11' is an open-pollinated cultivar developed in California. It has fair

yield performance. 'Tainan No. 3' was introduced from Taiwan; it is among one of the poorest yielding cultivars at both locations.

The other three of the eight plant materials were chosen from the 1989 M. S. U. Asparagus Clone Trial, conducted at the same two locations. These clones were developed by selecting outstanding asparagus crowns in commercial plantings and then multiplying by micropropagation. Plants of each clone have the same genotype and their characters should be highly homogeneous. The three clones used in this study are 'Hart-3', '86Ram-3' and '86Sam-3'.

At each location for each of the eight entries, two male plants were selected; efforts were made to have these two plants similar in stalk number and growth habit. Tables 1 and 2 summarize the characteristics of the eight cultivars and lines at these two locations.

I observed the growth habit of these eight cultivars and lines and measured the average height of five plants of each of the eight cultivars at each location (Tables 1 and 2). In the two locations, '44Px22-8' had a very vigorous growth with dense fern and lateral branches entangling with each other. 'Jersey Giant' had a medium-dense fern growth and a fair population density. 'Franklim' had a short plant type with a very concentrated and heavy fern growth. 'UC86-11' had a dense population and a vigorous fern growth. 'Tainan No. 3' had a relatively poor population establishment and relatively sparse fern growth. Of the three clones, growth habit of 'Hart-3' was similar to that of '44Px22-8'. It had a very dense fern growth and good population establishment. '86RAM3' had a small plant type and not a very vigorous growth. '86SAM3' had a medium population density with a relatively sparse fern growth

Table 1. Plant information of cultivars and lines sampled at SWMREC.

Cultivar	Sourse	Cultivar type	Yield of 1995 (pounds/acre)	Average yield of six harvests (pounds/acre)	Average height of 5 plants (cm)
44Px22-8	Rutgers	all-male hybrid	6453	4834	202
Jersey Giant	Rutgers	all-male hybrid	5637	4178	183
UC86-11	California	open-pollinated	4148	3330	196
Franklim	Netherlands	all-male hybrid	4920	3164	162
Tainan No.3	Taiwan	open-pollinated	2281	1502	173
Clones	**Source**			**Average yield of four harvests (pounds/acre)**	
Hart-3	M.S.U	all-male clone	6565	4199	186
86Sam3	M.S.U	all-male clone	2952	1878	194
86Ram3	M.S.U	all-male clone	2500	1726	162

Table 2. Plant information of cultivars and lines sampled at Hart MI.

Cultivar	Sourse	Cultivar type	Yield of 1995 (pounds/acre)	Average yield of six harvests (pounds/acre)	Average height of 5 plants (cm)
Franklim	Netherlands	all-male hybrid	5439	4575	167
44Px22-8	Rutgers	all-male hybrid	4024	3724	190
Jersey Giant	Rutgers	all-male hybrid	3862	3356	200
UC86-11	California	open-pollinated	2679	2307	180
Tainan No.3	Taiwan	open-pollinated	2046	1425	178
Clones	**Source**			**Average yield of four harvests (pounds/acre)**	
Hart-3	M.S.U	all-male clone	7381	5669	210
86Ram3	M.S.U	all-male clone	5397	4102	179
86Sam3	M.S.U	all-male clone	686	562	190

B: Field measurement.

An open system of measuring asparagus whole-plant gas exchange rate developed in Dr. James Flore's lab was used. A whole asparagus plant was enclosed in a plastic tent which functioned as an assimilation chamber. When using an open system to measure gas exchange rate, there is a net flow of air through the system. In our case, air was blown into the assimilation tent by a blower at the bottom of the tent. An outlet in the top of the tent allowed the outflow of the air. Thus, a steady flow of air through the tent was maintained.

The plastic material used to make the assimilation tent was 'Mylar', a polyester film. (DuPont, Wilmington, Del.). 'Mylar' has very high light transmission property, an average of 87% transmission of visible light.

The tent was made about 300 cm tall, considering that the highest plant in our experiment was about 220 cm. Three pieces of Mylar in rectangular shape, each of 3 m long and 1m wide, were sealed into a cylinder-shape using a strong transparent tape. Another piece of 'Mylar' was used to cap the top. The height of the assimilation tent was three meters and the perimeter was 2.7 meters. An opening was made on the top of the tent as an air outlet.

Field measurement of photosynthetic rate was done as follows.

- 1) Foam rubber was wrapped around the bases of stalks so that a mat structure was formed next to the ground.
- 2) The assimilation tent was put over the plant.
- 3) The bottom part of the tent was collected and tied tightly around this mat structure so that the plant gas exchange system was isolated from the soil respiration system, this was to minimize the effect of soil respiration on plant gas exchange.
- 4) A fan was turned on to blow air into the tent through a pipe connected at the bottom of the tent. Within minutes, the tent inflated to its full extent and air flow was detected exiting of the outlet at the top. The pipe was about 1.3 m long and had a

diameter of 8 cm. There was a hole in the middle of the pipe so that a tube could be inserted for the measurement of reference CO_2 concentration of the environment and air flow speed. Flow speed can be controlled at the fan-end of the pipe by adjusting the openness of this end.

- 5) A tube was put into the tent through the outlet on the top, and this tube was connected to the inlet port of an air pump, the pump functioned to withdraw sample air from the top of the tent.
- 6) Another tube started at the outlet port of the pump and was connected to a leaf chamber; The leaf chamber, which contained a humidity sensor and a light sensor, was connected to a portable infra-red gas analyzer (IRGA), (model LCA2; Analytical Development Co., Hoddesdon, U.K.). The leaf chamber is so-called because it contains a chamber for single leaf photosynthetic rate measurement, which was not relevant to this experiment. The humidity and light sensors on it were used in this experiment.

Through this connection system, a flow of sample air was drawn from the top of the tent and drawn through the leaf chamber before reaching the gas analyzer. The leaf chamber was connected to the IRGA by a wire at the " Auxiliary signal input & output" socket in the analyzer.

The parameters that can be measured by the IRGA are as follows.

- 1) Reference CO_2 concentration.
- 2) Relative humidity, inside and outside of the assimilation tent.
- 3) Temperature of the plant in $^{\circ}C$.
- 4) Photosynthetic active radiation (PAR) in $\mu mol\ m^{-2}\ s^{-1}$.
- 5) Differential CO_2 in vpm, which was the indication of the amount of CO_2 assimilated by the canopy.

There are two selectors on the top of IRGA; the 'display selector' can point to the following selections: CO_2 measurement, %RH, $^{\circ}C$, and PAR. The 'mode selector' is only

for CO_2 measurement; it functions only when 'display selector' points to 'CO_2 measurement'. The measuring process was as follows.

- 1) Turn on IRGA by turning the 'display selector' from 'off' to 'CO_2 measurement', then switch the 'mode selector' to 'ref', the screen displays reference CO_2 concentration.
- 2) Switch the 'display selector' to '%RH'; the screen displays inside RH; then open the leaf chamber, this allows the outside air to flow through the leaf chamber to the IRGA; the screen then displays the outside RH .
- 3) Switch the 'display selector' to '°C'; the screen displays temperature.
- 4) Switch the 'display selector' to 'PAR' and hold the leaf chamber to allow the light sensor to face the sun; record the maximum number reached.
- 5) Switch the 'display selector' to 'CO_2 measurement' again, and switch the 'mode selector' to 'DIFF' which is the mode for measuring the differential CO_2 concentration. It generally took 3 to 4 min to reach some steady readings; usually about five readings were taken for each plant measurement.
- 6) A flow meter was used to measure the flow speed (m $s^{-1)}$ of the air blowing into the tent. After these measurements, the system was disassembled and moved to measure the next plant. It took about half an hour to complete the measurement of one plant. The time of day when measuring each plant and the weather condition at the time of measurement were recorded.

The last harvest in SWMREC was at June 16 and last harvest in Hart was June 19, 1995. The first measurement at SWMREC was on July 25, 1995 and on July 28, at Hart. By the time we began the field measurement, most of the fern had grown to its maximum size. The above-ground portion did not increase significantly after the end of July. After the last measurement in September, every plant measured was cut at the ground level to obtain its dry weight. Each plant was separated into stem portion and fern portion; stem and fern were weighed separately after 48 hours in a 50°C-drying oven. The mass of each plant so measured was taken as its assimilating mass during the whole season. It was not

feasible to cut the plant to have dry weight after each measurement. During the experiment there were sporadically a few spears coming out of the ground; they were eliminated so that the mass of the existing assimilation organ could be estimated. A spear emerging late in the season and growing into fern was likely not be able to contribute photosynthetically as much as it consumed during growth.

Leaf area was measured by using a Li-Cor Portable Area Meter (Model LI-3000). Three replications were measured for each genotype. Leaf area ratio (LAR, leaf area per gram dry weight) was calculated by dividing leaf area by dry weight. Total leaf area of each individual plant was calculated by its multiplying LAR by its fern dry weight. Leaf area of asparagus is more difficult to estimate in comparison with area estimates of broad leaf plants, because of the cylindrical shape of its cladophyll. Observations indicate that the leaf areas of asparagus probably were underestimated in this study due to extensive and unavoidable overlapping of cladophylls. Even though this does not affect the comparison of photosynthetic rates of the eight genotypes used in this experiment, the absolute value of photosynthetic rates expressed on a leaf area basis probably are greater than the true values.

C: Experimental design of the field measurement.

In field measurement, it took about half an hour to measure one plant. This meant that one hour was needed to finish the measurement of one genotype. Because of this, the biggest concern in field measurement is the time-of-day effect on photosynthetic rate measurement. This effect can be reduced by measuring genotypes in a rotating sequence during the whole season. The designs of such an order of measurement during the whole season at the two locations are shown in Tables 3 and 4 .

Concerning the statistical design, this experiment adopted a split-plot design. The main-plot was the eight genotypes at each block. The two locations were used as two

Table 3. Order of field measurement.
Location: SWMERC.

Group A Measurement	Order 1	2	3	4	Group B Measurement	Order 1	2	3	4
1st	Hart-3	86Ram-3	86Sam-3	Franklim	1st	Jersey Giant	UC86-11	Tainan-3	44Px22-8
2nd	86Ram-3	86Sam-3	Franklim	Hart-3	2nd	UC86-11	Tainan-3	44Px22-8	Jersey Giant
3rd	86Sam-3	Franklim	Hart-3	86Ram-3	3rd	Tainan-3	44Px22-8	Jersey Giant	UC86-11
4th	Franklim	Hart-3	86Ram-3	86Sam-3	4th	44Px22-8	Jersey Giant	UC86-11	Tainan-3
5th	Hart-3	86Ram-3	86Sam-3	Franklim	5th	Jersey Giant	UC86-11	Tainan-3	44Px22-8
6th	86Ram-3	86Sam-3	Franklim	Hart-3	6th	UC86-11	Tainan-3	44Px22-8	Jersey Giant
7th	86Sam-3	Franklim	Hart-3	86Ram-3	7th	Tainan-3	44Px22-8	Jersey Giant	UC86-11

Table 4. Order of field measurement.
Location:Hart MI.

Group A Measurement	Order 1	2	3	4	Group B Measurement	Order 1	2	3	4
1st	Franklim	86Ram-3	86Sam-3	Hart-3	1st	UC86-11	Jersey Giant	44Px22-8	Tainan-3
2nd	86Ram-3	86Sam-3	Hart-3	Franklim	2nd	Jersey Giant	44Px22-8	Tainan-3	UC86-11
3rd	86Sam-3	Hart-3	Franklim	86Ram-3	3rd	44Px22-8	Tainan-3	UC86-11	Jersey Giant
4th	Hart-3	Franklim	86Ram-3	86Sam-3	4th	Tainan-3	UC86-11	Jersey Giant	44Px22-8
5th	Franklim	86Ram-3	86Sam-3	Hart-3	5th	UC86-11	Jersey Giant	44Px22-8	Tainan-3
6th	86Ram-3	86Sam-3	Hart-3	Franklim	6th	Jersey Giant	44Px22-8	Tainan-3	UC86-11
7th	86Sam-3	Hart-3	Franklim	86Ram-3	7th	44Px22-8	Tainan-3	UC86-11	Jersey Giant

blocks. The sub-plot was the seven measurements through the season for each genotype. The total variation was composed of the variation caused by (i) the genotypic differences on photosynthetic rate, (ii) the measuring date effect, which reflected the seasonal change of photosynthetic rate, (iii) the interaction between main-plot and sub-plot.

D: Calculation of the data from field measurement.

Photosynthetic rates were calculated by using a BASIC computer program written by Moon and Flore (1986). This program is for the calculation of photosynthetic rate measured in an open gas exchange system.

The calculation of each field measurement was begun by setting up a simple file which contains: the "plant I.D.", leaf area (dm^2), relative humidity inside and outside the tent, plant temperature ($^{\circ}C$), ambient CO_2 concentration, and the delta CO_2 value reading from IRGA as a positive number. For example: " 1, 07, 25",162,64,50,29.5,334,12, which contains, in sequence, the above information. A file name was given to this INPUT file. The BASIC program was started to process these data, and the INPUT file was entered. The program asked for two other parameters for calculation, which were flow rate in liters $\cdot$ min^{-1} and PAR value in μmol m^{-2} $\cdot s^{-1}$. The PAR value was from the field measurement. The flow rate was derived from the flow speed value ($m \cdot s^{-1}$). The program asked for an output file name when calculations were completed. This output file contained the result of calculations including vapor pressure deficit (VPD), net CO_2 assimilation rate (A), stomatal conductance (gs), transpiration rate (E), and intercellular CO_2 concentration.

Part two: Laboratory study.

A: Sample preparations.

Cladophyll samples of the eight asparagus genotypes were collected from the field in early September 1995; efforts were taken not to include any branches in the sample. Samples, about 50 gram for each genotype, were bagged in aluminum foil and stored at -80°C until need and never allowed to thaw. The preparation of samples was done in a cold room (2.5°C). Frozen fern material, about 2 grams per replication, was ground in a mortar previously cooled with liquid nitrogen; The powder was transferred to a disposable vial containing 500 mg (5%, w:v) insoluble polyvinylpoly pyrrolidone (PVPP). PVPP can bind plant polyphenols to prevent their oxidization and polymerization; polymerized polyphenols can bind to and inactivate proteins. To the vial, 10 ml degassed buffer was added (100 mM Tris-HCI, pH 8.3, 20mM $MgCI_2$, and 10 mM DTT[added just prior to use]). DTT prevents enzyme oxidation. The ground tissue was homogenized using a Polytron at its full speed(2x15s). The solution was transferred to a graduated tube, and the crude volume of the solution was recorded. For chlorophyll determinations, three subsamples each of 500 µl crude extracts were stored at -80°C until further analysis. For rubisco assay, the crude solution was filtered through a layer of Polycloth and centrifuged at 20,200xg for 10 minutes. The supernatant was transferred into a graduated tube, and the extraction solution volume was measured and recorded. This extraction solution tube was kept on ice prior to assaying for rubisco.

B: Rubisco activity assay.

B.1: Reagents.

(i) RuBP, 5 mM. (Sigma St.Louis). (ii). Carboxylase buffer, CO_2-free 100 mM Tris-HCl, pH 8.3, 20mM $MgCl_2$, and 10 mM DTT. This is the same buffer used in the preparation of samples. (iii) [^{14}C]$NaHCO_3$, A 0.2 M $NaHCO_3$ solution is prepared, a known amount of ^{14}C is added prior to use. The specific activity in mCi/µmol is

calculated for each assay (see below). (iv) Rubisco, prepared and purified by the above described process was contained in the extraction solution.

B.2: Optimization of asparagus rubisco assay.

In order to run an assay successfully, the assay process needs to be optimized. Assay of asparagus rubisco was optimized in the following aspects.

- 1) Desalting according to the method of Neal and Florini (1973). Extraction solution was added on the top of a Sephadex G-25 column equilibrated with desalting buffer (100 mM Tris-HCl, pH 8.6, 20 mM $MgCl_2$ 10 mM $NaHCO_3$, 1mM dithiothreitol). The column was centrifuged for two minutes, and the eluate was collected for assay. The addition of $NaHCO_3$ and $MgCl_2$ in the desalting buffer was to see whether their presence would activate the enzyme.
- 2) Activation prior to assay. Asparagus rubisco was activated by preincubation with CO_2 and Mg^{++} for five min before adding RuBP to initialize the reaction. This treatment was compared with the control to see if the activation process can enhance enzyme activity.
- 3) Linearity of rubisco activity with time was studied by terminating the reaction at 0, 30, 60, 90, 120 seconds.
- 4) Four concentrations of RuBP, 0.25, 0.5, 1, and 2 mM were compared to determine the optimum concentration for asparagus rubisco activity.
- 5) Three different amount of purified rubisco solution, 25, 50, and 75 μl, were compared to determine the optimum amount of enzyme solution which can maximize the reaction.

Cladophyll samples of the genotype Franklim was used in the optimization process; it was assumed that its conditions would be optimal for the other two genotypes.

B.3: Rubisco assay procedure.

The assay was conducted under a fume hood according to the method of Lorimer et al. (1977). The total volume of reaction solution in each glass tube (13x100mm) was 500 µl. The procedure is as follows:

- 1) To each tube, add 350 µl extraction buffer.
- 2) Then add 50 µl [^{14}C]$NaHCO_3$ solution; shake the tubes to mix it well and put them on a 30°C heatblock.
- 3) Next add 50 µl extraction solution which contains the rubisco to be assayed.
- 4) Start the assay by adding 50 µl RuBP;
- 5) Inject 300 µl acetic acid to stop the reaction at appropriate time.

Each of the above components was added from a 10x concentrated stock solution. The final concentration in the reaction solution was one tenth of the stock solution concentration. Samples were then heated at 100°C for five min. This was done to drive off the unfixed $^{14}CO_2$ and to leave acid-stable products. The contents of each tube were then transferred to scintillation vials, and samples were counted in a liquid scintillation counter (Packard 1500). Aliquots of the bicarbonate stock solution also were counted to determine their specific activities. Each vial was counted three times. The means of the three was used to calculate the rubisco activity (see below).

B.4: Rubisco activity calculation.

For each assay, the specific activity of radioactive bicarbonate was calculated first; the activity in each sample assay tube was based on the specific activity of stock bicarbonate solution. The calculation of the specific activity of stock bicarbonate solution is shown in the following example:

- 1) In one assay the average DPM value of the three stock bicarbonate solution was 87,671 dpm/5 µl; 5 µl was the amount added.
- 2) In each rubisco assay tube, 50 µl stock solution was added; the total activity would be 876,710 dpm per assay.

- 3) The mole concentration of the $NaHCO_3$ stock solution was 0.2 M, which was 16.8 mg ml^{-1}. As we added 50 µl each assay, by calculation there was (16.8/1000)*50=0.84 mg $NaHCO_3$/assay; divided by the molecular weight of $NaHCO_3$ it gave 10 µmol $NaHCO_3$ in each assay. So, the specific activity of radioactive bicarbonate was 87,671 dpm/µmol . As 1 µCi=$2.22x10^6$ DPM, this equals to 0.0395 µCi/µmol.

The activity in each assay tube was calculated using the above specific activity value and the background subtracted DPM values, Table (5) shows the calculation of a time course CO_2 fixation assay.

Table 5. Calculation of rubisco activity.

seconds	DPM	µmol/min
30	2284	0.052
60	4526	0.052
90	7714	0.059
120	9849	0.056

The average of the four values, 0.05475 µmol/min/assay, indicated the rubisco activity of this assay, and it can be interpreted that 0.05475 µmol $^{14}CO_2$ was fixed in a minute.

C: Chlorophyll determination.

Chlorophyll content was determined according to the method of Wintermans and De Mots (1965). Briefly this involved extracting chlorophyll from the plant material in 96% ethanol. Crude extract of 40 µl was mixed with 960 µl absolute ethanol and allowed to extract for 15 min on ice under subdued light, the samples were vortexed every 5 min. Then extracts were centrifuged at 13,000g for 5 min at room temperature, and the absorbency of the supernatant was measured in a Hitachi U3110 spectrophotometer (Hitachi Danbury, CT) using 96% ethanol as a blank. Absorbency was measured at three wavelengths: 649 nm, 665 nm and 730 nm. The chlorophyll content (mg) in 40 µl crude solution sample was calculated by following formulas:

- 1) Chlorophyll a=13.7 x (Abs665-Abs730) - 5.78 x (Abs649-Abs730).
- 2) Chlorophyll b=25.8 x (Abs649-Abs730) - 7.6 x (Abs665-Abs730).
- 3) Total chlorophyll= 20.04 x (Abs649-Abs730) + 6.1x (Abs665-Abs730).

The replication number for the chlorophyll determination of each genotype was five.

D: Protein determination.

Total soluble protein content of asparagus fern was determined using the Bradford (1976) method with BSA (Bovine Serum Albumin) as the standard. Aliquots of the supernatant used in the enzyme assays (see above) were diluted, and its absorbency was measured at 595 nm using a Hitachi U3110 spectrophotometer. The total soluble protein content of the sample was determined by comparing its absorbency to the absorbency of the BSA standard.

Chapter three: Results and discussion.

Part One: Diurnal variation of asparagus photosynthetic rate.

One of the important aspects of photosynthetic activity of asparagus is its daily change pattern. Daily photosynthetic activities were studied on two sunny days during August, 1995.

At SWMREC, on August 13, 1995, photosynthetic rate of one plant of the cultivar Franklim was measured ten times from 9 AM to 6 PM, once every hour. Figure 1 shows the daily changes of photosynthetic rate and temperature. Figure 2 shows the daily changes of environmental CO_2 concentration and photosynthetically active radiation (PAR). On the same date and at the same location, photosynthetic rate of one plant from the cultivar Jersey Giant also was measured ten times from 9:30 AM to 6:30 PM at an one-hour intervals. Figures 3 and 4 show the daily photosynthetic rate change and three other parameters of this plant.

As shown in Figure 1, photosynthetic rate of the 'Franklim' plant increased from 23.9 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at 9 AM, the first measurement, to 32.6 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at 10 AM. In the following three hours, photosynthetic rates changed very little; the rates were 33.3 at 11 AM, 31.6 at 12 PM, and 33.7 at 1 PM; the rate at 1 PM was the peak for the day. After reaching the peak, there was a large decrease to 22.4 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at 2 PM. From 2 PM to 6 PM, the trend was a gradual decrease with rates of 23.2 at 3 PM, 20 at 4 PM, 17.1 at 5 PM, and 19.5 at 6 PM. Table 6 shows the daily changes of vapor pressure deficit (VPD), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) of this plant.

Figure 1. Daily changes of photosynthetic rate and temperature. 08/13/95; SWMREC; Cultivar: Franklim

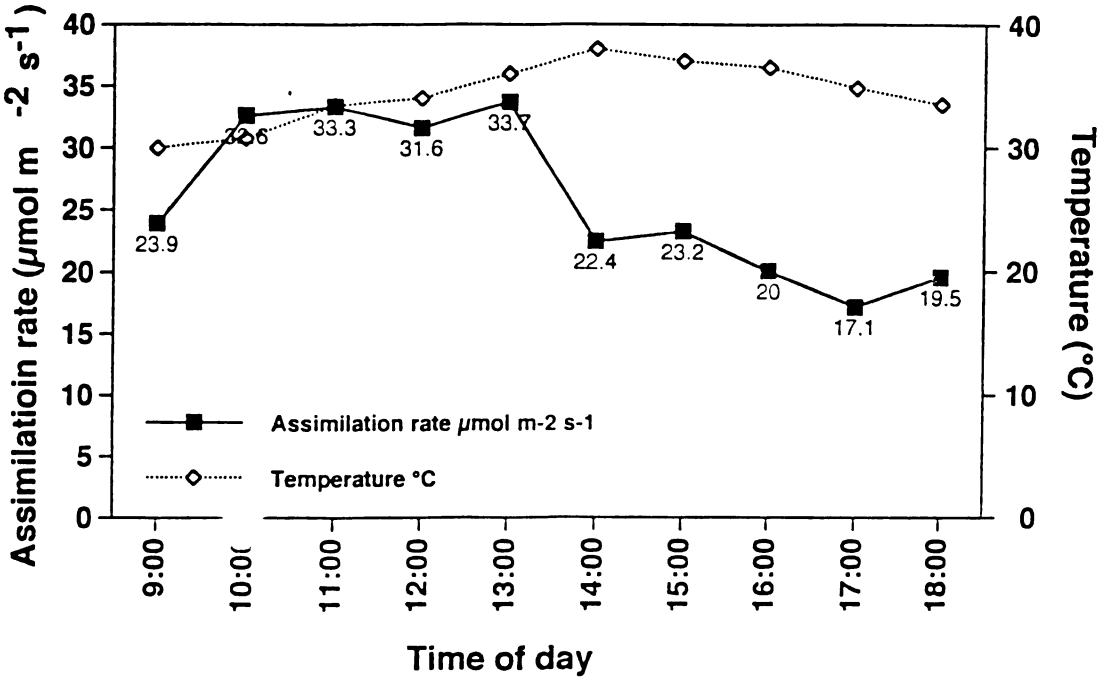


Figure 2. Daily changes of PAR and reference CO_2 concentration. 08/13/95; SWMREC; Cultivar: Franklim

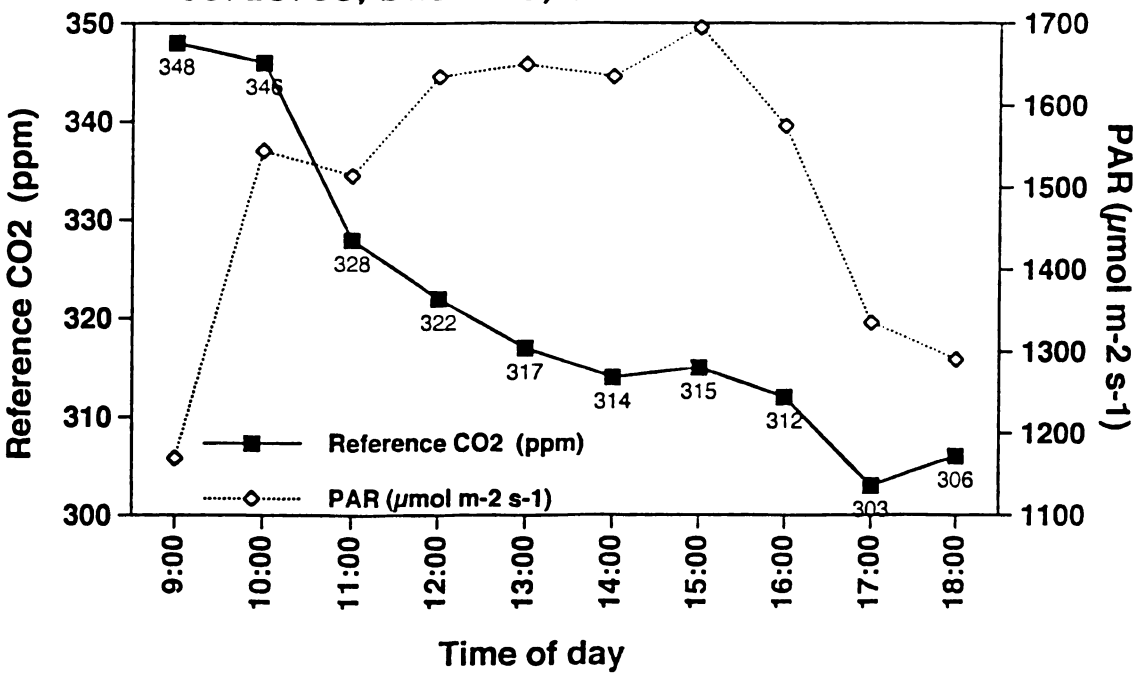


Figure 3. Daily changes of photosynthetic rate and temperature. 08/13/95; SWMREC; Cultivar: Jersey Giant

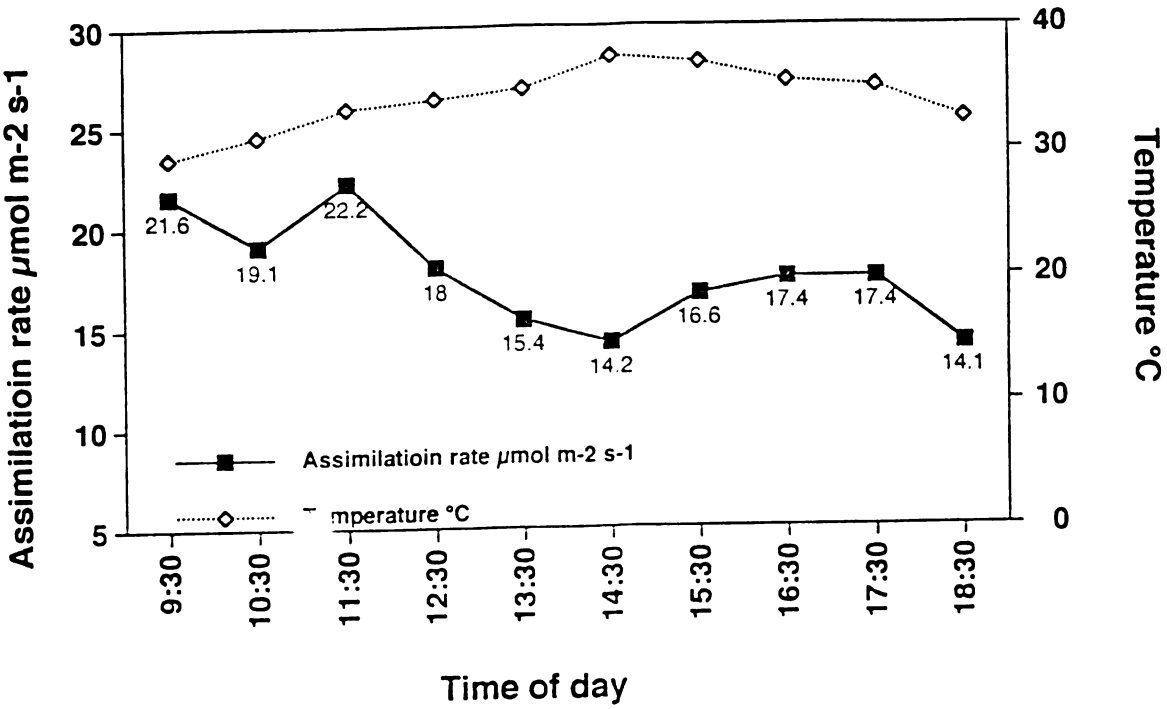


Figure 4. Daily changes of PAR and reference CO_2 concentration. 08/13/95; SWMREC; Cultivar: Jersey Giant

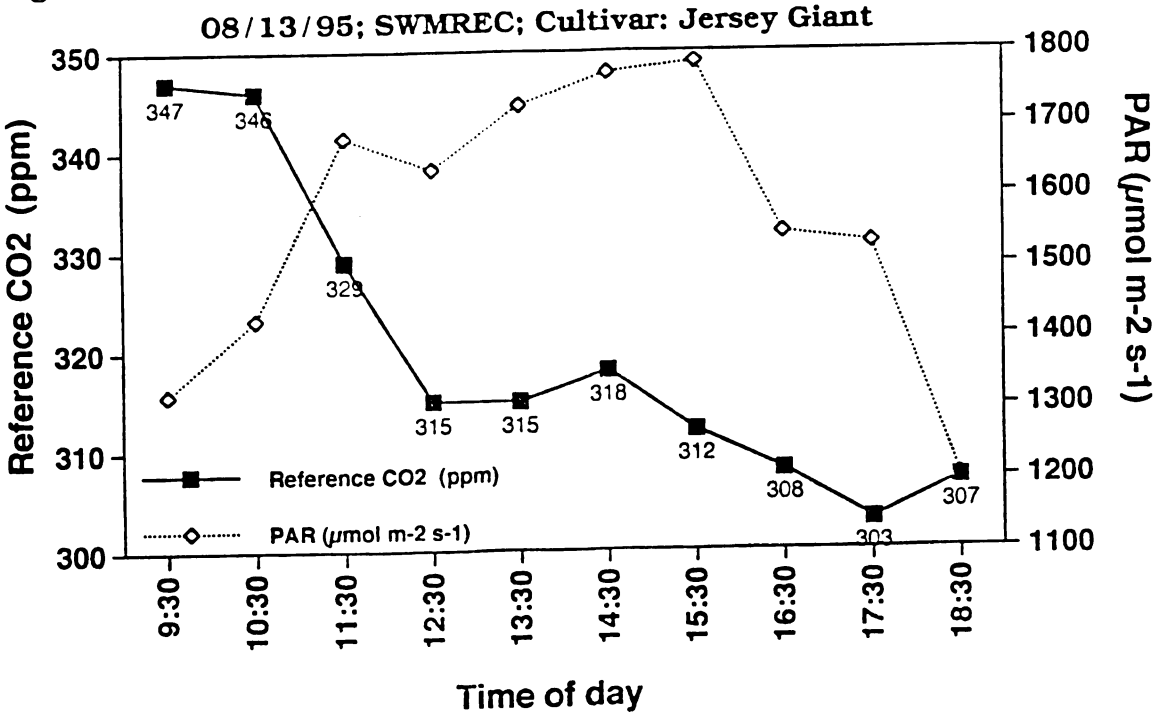


Table 6 Daily changes of vapor pressure deficit, stomatal conductance, and intercellular CO_2 concentration of the cultivar Franklim on August 13, 1995 at SWMREC.

Time	VPD (KPa)	g_s ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Ci ($\mu mol\ CO_2 \cdot mol^{-1}$ CO2)
		1050.1	365.1
9:00 AM	0.94		
10:00 AM	0.22	1100.5	374.2
11:00 AM	0.26	2137	311
12:00 PM	0.81	911.4	283.2
1:00 PM	1.44	515.7	244.9
2:00 PM	2.34	196.9	190.2
3:00 PM	2.22	398.5	246.3
4:00 PM	2.4	185.7	194.0
5:00 PM	2.13	75.1	68.5
6:00 PM	1.78	206.1	203.7

Closely related to the drop of photosynthetic rate between 1 PM and 2 PM was a sharp decrease of g_s, (from 515.7 μmol · m^{-2} · s^{-1} at 1 PM to 196.9 μmol · m^{-2} · s^{-1} at 2 PM). During this time, intercellular CO_2 concentration dropped from 244.9 to 190.2 μmol CO_2/mol CO_2. VPD increased greatly after 1 PM, which caused the closure of stomata in order to reduce water loss. The decrease in photosynthetic rate after 1 PM can be explained largely by these changes in VPD, g_s, and Ci.

Based on the above, daily photosynthetic rate changes of 'Franklim' at SWMREC can be divided into four periods.

- 1) A period of starting and increasing from early in the morning to about 10 AM.
- 2) A period of stable, active photosynthesis with high photosynthetic rate from 10 AM to 1 PM.
- 3) A period of gradually decreasing, less active photosynthesis from 2 PM to 6 PM.
- 4) A period of decreasing photosynthetic rate after 6 PM until photosynthesis completely stops.

Daily changes of environmental CO_2 concentration are shown in Figure 2. It was highest at 9 AM (348 ppm). This was likely due to the accumulation of CO_2 at night resulting from lack of CO_2 consumption and release of CO_2 by plant respiration. From 9 AM to 2 PM, environmental CO_2 concentration dropped abruptly; it was 314 ppm at 2 PM, a 34 ppm drop within 5 h. This was the period of very active photosynthesis. The rate of decrease levelled after 2 PM, and the lowest environmental CO_2 concentration was 304 ppm at five PM. The daily change of environmental CO_2 concentration reflected the photosynthetic activity of asparagus plants. It was highest early in the morning when photosynthesis just started. Consumption of CO_2 by active photosynthesis during the day caused its concentration to decrease rapidly. Low CO_2 concentration in the afternoon might be a rate-limiting factor for asparagus photosynthesis and might be responsible, combined with decreasing stomatal conductance, for the decrease in photosynthetic rate in the afternoon.

The pattern of environmental temperature change (Figure 1) was simple. It was increasing between 9 AM and 1 PM and then decreasing between 1 PM and 6 PM. The lowest temperature was at 9 AM (30°C); the highest was at 1 PM (38°C). There was no evidence to indicate that such high environmental temperature had any negative effect on asparagus photosynthesis. Two previous studies on asparagus photosynthesis reported that the best temperature for asparagus photosynthesis is about 20°C. (Sawada et al., 1965; and Lin et al., 1978). Both studies were carried out under controlled indoor conditions and measured photosynthetic rate of asparagus twigs in assimilation tubes. Thus, the degree such conclusions about optimum temperature for asparagus photosynthesis can reflect the situation in field is very questionable. Active photosynthetic activity for asparagus mostly occurred during July and August in Michigan. High temperatures above 30°C are not unusual during these two months. It is reasonable to conclude that 'Franklim' has adapted successfully to the high temperature growth condition during the summer in Michigan. In fact, it is logic that cultivars with good yield performance are able to manage active photosynthesis under high temperature conditions of Michigan summers.

Daily change of photosynthetic active radiation is shown in Figure 2. As the lowest PAR value was 1170 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (9 AM), it is obvious that PAR was not a factor limiting photosynthesis in this study. The light conditions of this day were sufficient for maximum activity of asparagus photosynthesis.

On the same date, daily photosynthetic rate changes of one plant of 'Jersey Giant' was measured (Figure 3). The pattern of the daily photosynthetic rate change for this plant generally agrees with that observed for 'Franklim'. There was active photosynthesis during the whole period of measurement from 9:30 AM to 6:30 PM. This period of active photosynthesis can be divided into two parts; from 9:30 AM to 1:30 PM was a period of active photosynthesis with a high average rate (20.2 $\mu mol \cdot m^{-2} \cdot s^{-1}$); from 1:30 PM to 6:30 PM was a period with a lower average photosynthetic rate (15.8 $\mu mol \cdot m^{-2} \cdot$

s^{-1}). The peak of the day was at 11:30 AM (22.2 μmol · m^{-2} · s^{-1}). The lowest rate during the first period, 18 μmol · m^{-2} · s^{-1} at 12:30 PM, was higher than the highest rate during the second period, 17.4 μmol · m^{-2} · s^{-1} at 4:30 PM. This supports such a division. The daily pattern of photosynthetic rate change can be described in a similar manner as that of 'Franklim'. The daily changes of VPD, g_s, Ci are shown in Table 7.

Similar patterns of daily VPD, g_s, and Ci changes existed for 'Jersey Giant' as for 'Franklim'. Shown in Table 6, VPD increased greatly after 12:30 PM and the g_s value decreasd greatly after 12:30 PM. The decreasing stomatal conductance caused a reduction in intercellular CO_2 concentration. These changes were largely responsible for the decrease of photosynthetic rate in the middle of the day. On the other hand, there were a few differences between the two cultivars with respect to the patterns of daily photosynthetic rate change.

- 1) The peak of photosynthetic rate for 'Franklim' was at 1 PM while the peak for 'Jersey Giant' was at 11:30 AM.
- 2) There was a clear turning point between the two active photosynthetic periods in 'Franklim' marked by a big drop of photosynthetic rate between 1 PM and 2 PM; the change from the first active photosynthetic period to the second in 'Jersey Giant' was gradual, with small drops of photosynthetic rate between 11:30 PM and 1:30 PM.
- 3) The difference between the average rate of the two periods was 7.3 μmol · m^{-2} · s^{-1} for 'Franklim'; the difference was 4.4 μmol · m^{-2} · s^{-1} for 'Jersey Giant'.
- 4) Within the second period in 'Jersey Giant' there was a slight increase of photosynthetic rate from 1:30 PM to 4:30 PM followed by a decrease to the lowest point of the day at 6:30 PM; while in the second period of 'Franklim', photosynthetic rate decreased gradually from 2 PM to 5 PM.

Table 7. Daily changes of vapor pressure deficit, stomatal conductance, and intercellular CO_2 concentration of the cultivar Jersey Giant on August 13, 1995 at SWMREC.

Time	VPD (KPa)	GS ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Ci ($\mu mol\ CO_2 \cdot mol^{-1}\ CO_2$)
9:30 AM	0.42	1218.6	362.3
10:30 AM	0.05	8279.7	343.4
11:30 AM	0.52	1298.2	309.1
12:30 PM	0.87	909.2	290.8
1:30 PM	1.65	467.4	273.9
2:30 PM	2.47	242.5	247.8
3:30 PM	2.53	201.7	218.2
4:30 PM	2.33	174.2	198
5:30 PM	1.99	81	81.2
6:30 PM	1.88	111.2	172.3

The daily changes of environmental CO_2 concentration, photosynthetic active radiation (PAR), and environmental temperature were very similar to those of 'Franklim', as the measurements were made on the same day.

In Hart, on August 22, 1995, daily photosynthetic rates of one plant from the cultivar Franklim was measured nine times from 10 AM to 6 PM, once every hour. Figures 5 and 6 show the pattern of daily photosynthetic rate change of this plant and the changes of temperature, environmental CO_2 concentration, and PAR. Different from the two situations at SWMREC, there wasn't a clear difference in photosynthetic rates between morning and afternoon. Photosynthesis proceeded at a steady, slightly-changed rate through the day. The peak of assimilation rate was reached at 11:00 AM (38.9 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Then it dropped to 28.7 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at 12:00 PM, after which the plant maintained a steady rate of photosynthesis all the way to 5 PM with rates 26 at 1 PM, 30.7 at 2 PM, 28.9 at 3 PM, 23 at 4 PM, and 28.2 at 5 PM. It dropped to the lowest point of the day at 6 PM (22.4 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Table 8 presents the information on daily changes of VPD, g_s, and Ci for this plant.

Figure 5. Daily changes of photosynthetic rate and temperature. 08/22/95; Hart MI; Cultivar: Franklim

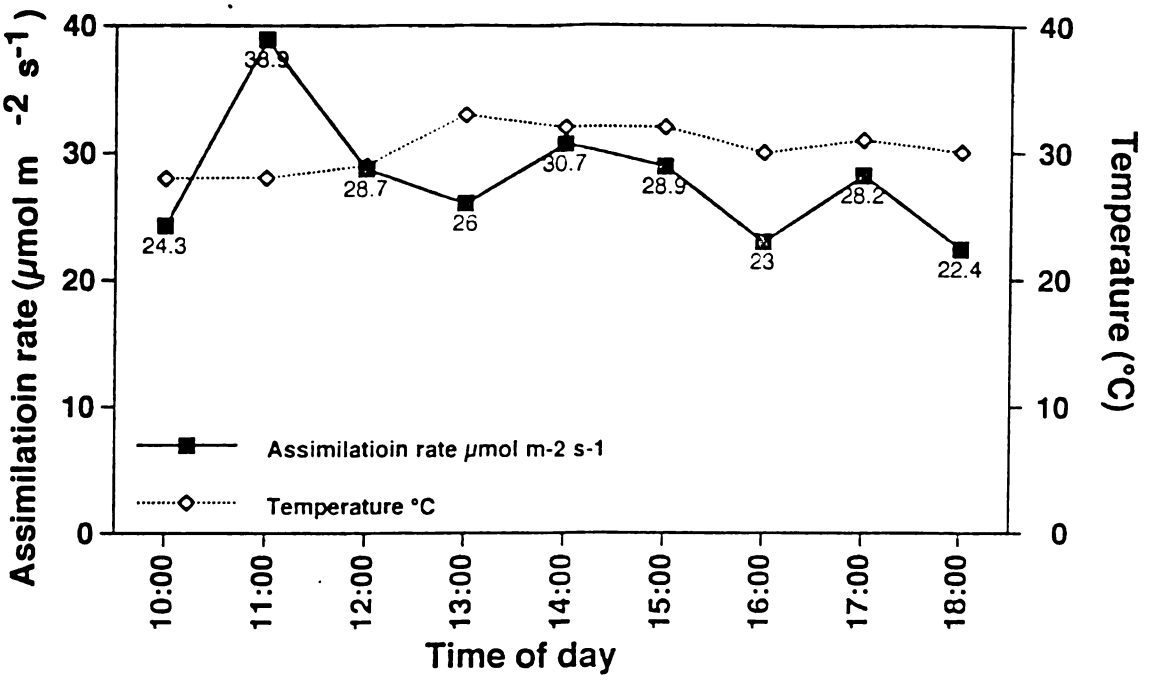


Figure 6. Daily changes of PAR and reference CO_2 concentration. 08/22/95; Hart MI; Cultivar: Franklim

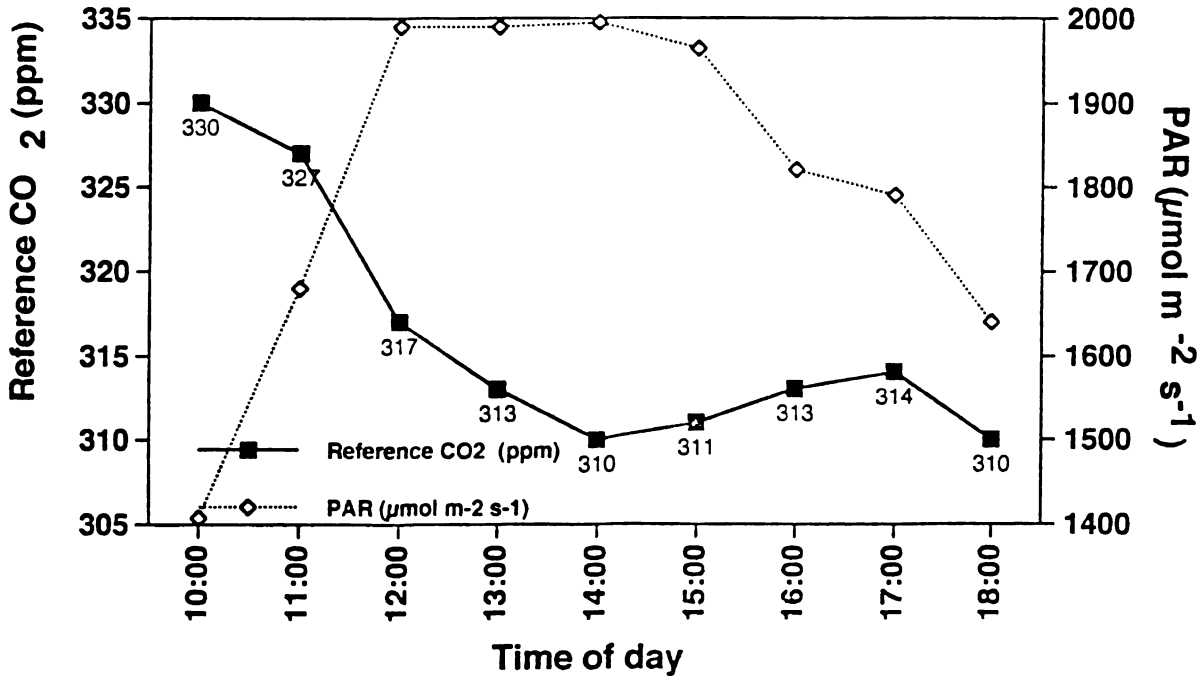


Table 8. Daily changes of vapor pressure deficit, stomatal conductance, and intercellular CO_2 concentration of the cultivar Franklim on August 22, 1995 at Hart.

Time	VPD (KPa)	GS ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Ci ($\mu mol\ CO_2\ mol^{-1}\ CO_2$)
10:00 AM	0.38	1078.6	305.5
11:00 AM	0.46	1020.4	286.5
12:00 PM	0.61	1455.4	294.2
1:00 PM	1.07	738.1	272.5
2:00 PM	0.96	1146.5	278.5
3:00 PM	1.06	551.6	253.6
4:00 PM	0.9	801.1	279.9
5:00 PM	1.32	161.5	134.4
6:00 PM	1.16	171.2	174.7

There was not as great an increase in VPD values between morning and afternoon for this plant at Hart on this day as for the two plants measured at SWMREC. The average VPD from 10 AM to 1 PM was 0.63 KPa, from 2 PM to 6 PM was 1.08, a difference of 0.45 KPa. Compared to the two cases at SWMREC: for 'Franklim', the average VPD before 1 PM was 0.73, after 1 PM was 2.17, a difference of 1.44; for 'Jersey Giant', average VPD before 1:30 PM was 0.7, after 1:30 was 2.24, a difference of 1.54. This clearly indicates that on August 22, there was not a great increase in vapor pressure deficit during that day. There also was no evident decrease in stomatal conductance during the day, until 5 PM. Intercellular CO_2 was maintained at a steady concentration during most of the day, until 5 PM. These differences in VPD, g_s, and Ci were the most likely reasons why this plant maintained a stable photosynthetic rate during the day.

From these studies, it seems there is not a universal daily photosynthetic rate change pattern that can be applied to different locations, different times in a season, different environmental conditions, or different cultivars.

The pattern of daily asparagus photosynthetic rate change during August on a sunny day can be depicted as such: it begins to increase after sunrise with increasing temperature and PAR with the presence of high environmental CO_2 concentration; this increase reaches a high point between 9 AM and 10 AM, and is followed by a period of active photosynthesis at a high rate for a few hours to about 1 PM; the peak of photosynthetic rate during the day is within this period at anywhere from 11:30 AM to 1 PM. Then, photosynthetic rate drops to a lower level; this decrease is primarily due to a reduction of stomatal conductance combined with other factors such as decreasing environmental CO_2 concentration, increasing temperature, and possibly light inhibition. In the afternoon, photosynthesis is carried out at this lower rate until it decreases to the lowest point after 5:00 PM.

Evidences presented here indicate that a very prominent factor limiting photosynthetic rate is CO_2 diffusion resistance, especially stomatal conductance (g_s).

Data on the changes of stomatal conductance and intercellular CO_2 concentration make it clear that they play a direct role in the changes of asparagus photosynthetic rate.

Part Two: Seasonal variation of asparagus photosynthetic rate. .

Seasonal change pattern of asparagus photosynthesis is another important aspect of this study. Compared to previous indoor studies on asparagus photosynthesis, field measurement has the advantage of making possible such a seasonal study. However, seasonal study involves many measurements at different dates through the season. Thus, there are more variables that can influence the results. For example, photosynthetic rates measured on a cloudy day in July might be lower than what it could be if the day were sunny; this may lead to an incorrect interpretation of seasonal trends. For the reason that most measurements were conducted on sunny days, I believe that this factor may have exerted an influence, but it did not distort the whole picture significantly.

The other confounding factor was that no one cultivar was measured at the same time in a day for each of its measurements through the season, so each cultivar's seasonal assimilation rate change was confounded seriously by daily assimilation rate variation. Instead of looking at one cultivar's seasonal assimilation rate change, the average assimilation rate of all the cultivars measured at each date was used to represent the assimilation rate of asparagus plant on that date.

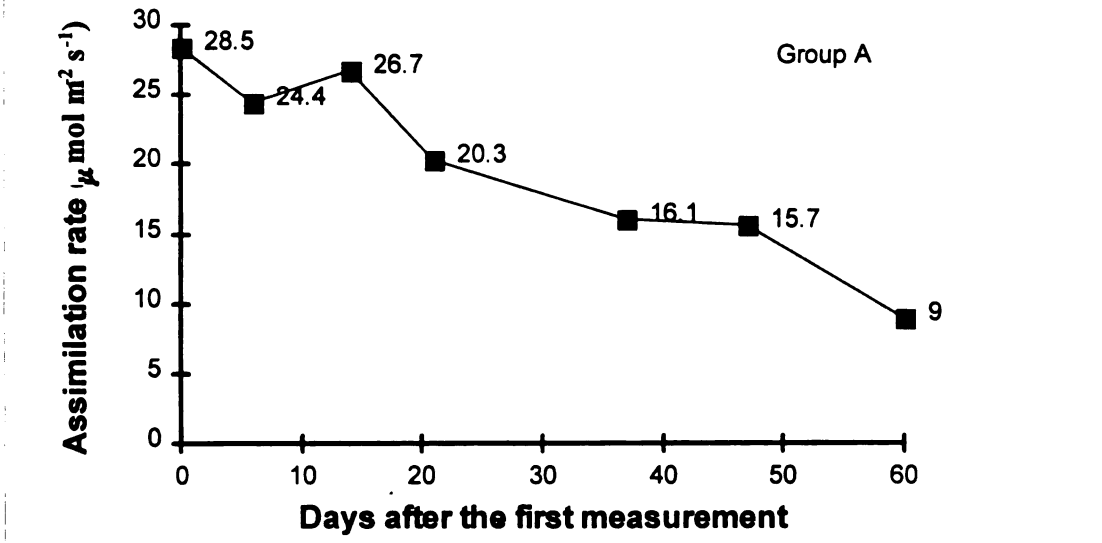
There were eight cultivars and lines in this study. At SWMREC, the eight were divided into two groups, because it was not always possible to measure all the eight cultivars within a day, generally four cultivars were measured on one day. Group A includes '86Ram-3', 'Hart-3', '86Sam-3', and 'Franklim'. These were measured on July 25 and 31; August 8 and 15; September 1, 2, 10, and 23. There was one cloudy day (July 25, the first measurement) and one partly sunny day (Aug. 8, the third measurement); all others were sunny days. The average assimilation rates of the four cultivars on each date were calculated; Figure 7a shows the seasonal change of photosynthetic rate of this group.

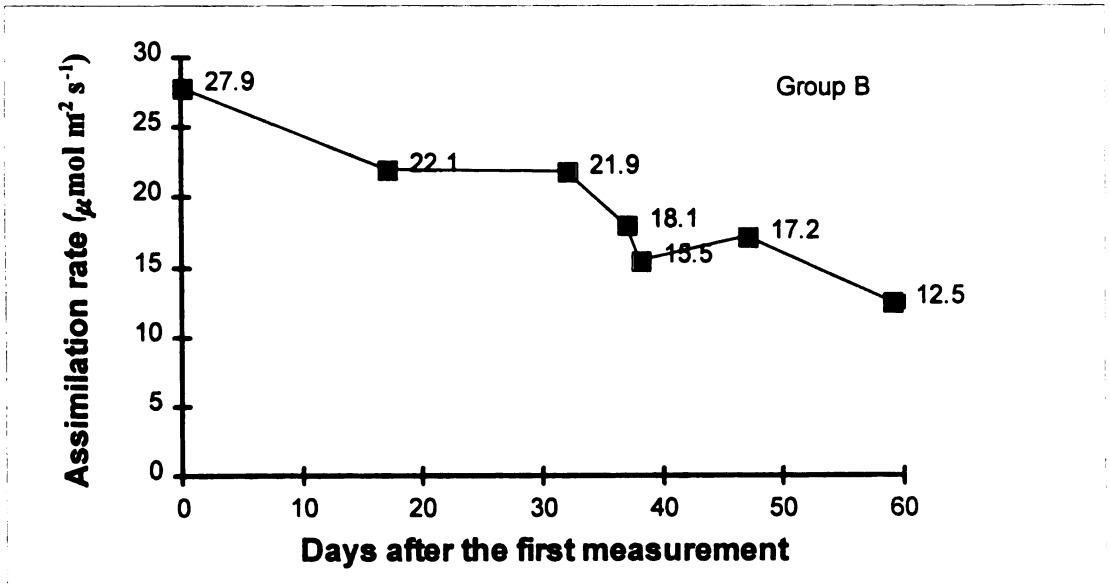
Group B includes: 'Tainan No. 3', 'Jersey Giant', 'UC86-11', and '44Px22-8'. These were measured on July 26; August 12, and 27; September 1, 2, 11, and 23. These

measurementsgg were made on sunny days, except for one cloudy day. Figure 7b shows the average assimilation rate of the four cultivars on each measuring day.

Figures 7a and 7b show a similar pattern of seasonal photosynthetic rate change. The highest rate was the first measurement in both groups: it was 28.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for group A measured on July 25, and 27.9 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for group B on July 26. Underground carbohydrate storage of asparagus likely was depleted greatly at the end of the harvest in the middle of June. By the end of July a vigorous fern growth was completed. In response to a high demand for carbohydrates by depleted sinks, ferns were photosynthesizing very actively at this time, evidenced by the high photosynthetic rate observed in this study. The average photosynthetic rate during August was 23.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for group A and 22 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for group B. The fact that photosynthetic rate maintained a high level in August indicates that asparagus plants were carrying out active photosynthesis during this month. August is the one month that contributes the most photosynthates. The fern did not grow to its full extent until middle to late July. Upon entering September, there was a trend toward decreasing photosynthetic rates. The average photosynthetic rate for group A in September was 14.2 $\mu mol \cdot m^{-2} \cdot s^{-1}$; it decreased to 9 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at the last measurement on Sept. 23, which was the lowest rate of all measurements. The average photosynthetic rate for group B in September was 15.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The lowest rate was obtained at the last measurement in Sept. 23 (12.5 $\mu mol \cdot m^{-2} \cdot s^{-1)}$. The ferns were in vigorous condition from July to the middle of September. The color of some cultivars began to turn yellow after the middle of September, and others began to turn yellow near the end of September. The change of fern color came along with the dropping of cladophylls to the ground. All these marked the end of the growing season. Unfavorable environmental

Figure 7. Seasonal changes of asparagus photosynthetic rate at SWMREC.
Assimilation rate for each date of Group A was the average of '86Ram-3', '86Sam-3', 'Hart-3', and 'Franklim'.
Assimilation rate for each date of group B was the average of 'Jersey Giant', 'UC86-11', '44Px22-8', and 'Tainan No.3'.
The seven measurements in group a were conducted on: 07/25, 07/31, 08/08, 08/15, 09/01, 09/10, 09/23.
The seven measurements in group B were conducted on: 07/26, 08/12, 08/27, 09/01, 09/02, 09/11, 09/23.





conditions and feedback mechanisms largely explain the decrease of photosynthetic rate in September. A significant amount of carbohydrates had beensynthesized and stored in crowns and storage roots after active photosynthesis from July to September. This exerted an end-product inhibition effect on fern photosynthesis, consistant with the widely accepted feedback theory. More and more unfavorable weather conditions including the shortening of day-length and decreasing temperature, also contributed to the decreasing photosynthetic activity.

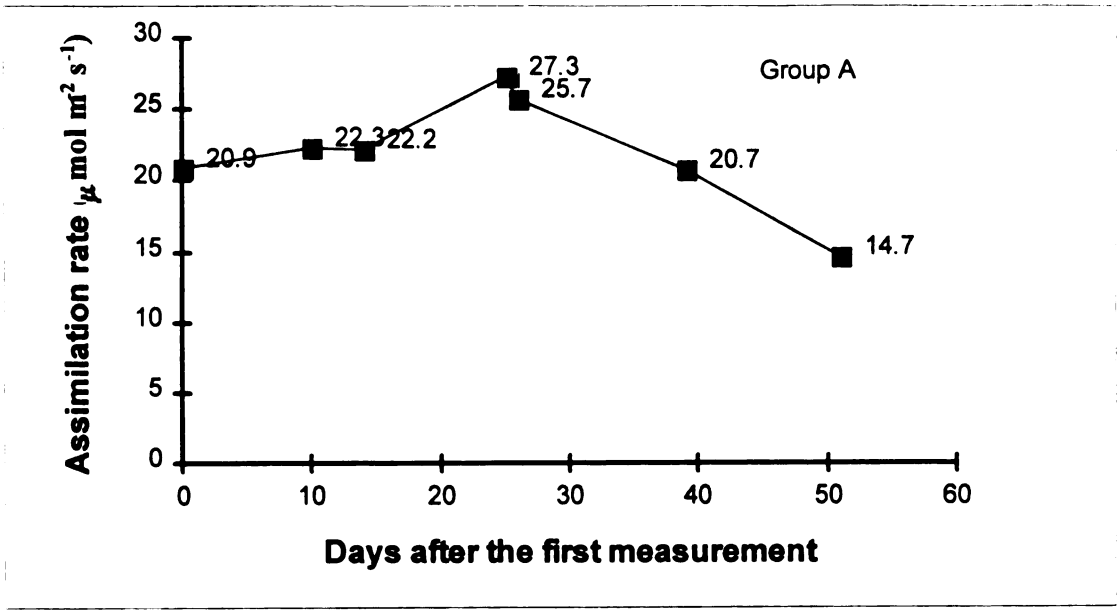
At Hart, as at SWMREC, the eight cultivars were divided into two groups. Group A includes: 'UC86-11', '44Px22-8', 'Jersey Giant', and 'Tainan No.3'. These were measured on July 27; August 6, 10, 21, and 22; September 4 and 16. During the above seven measurements, there was one cloudy day and one partly sunny day; the other five days were sunny.

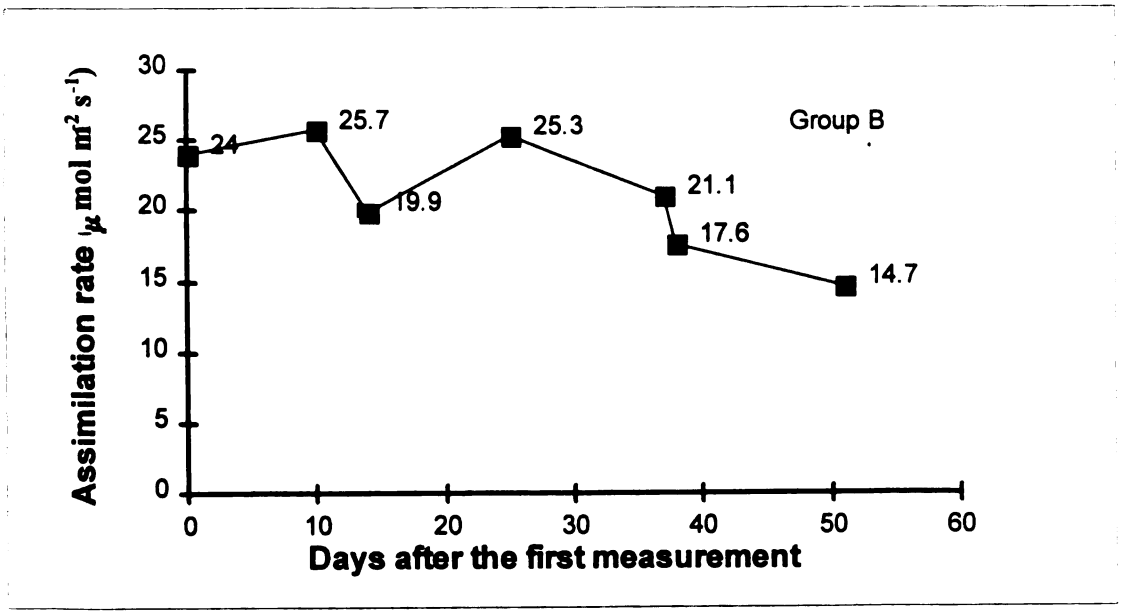
Group B includes: 'Hart-3', '86Ram-3', '86Sam-3', and 'Franklim'. These were measured on: July 28; August 7, 11, and 22; September 3, 4, and 16. Among these seven dates, there were one cloudy day, two partly sunny days, and four sunny days.

The average photosynthetic rate of four cultivars in each group were plotted against measuring days through the season. Figure 8 shows the seasonal trend of these two groups.

As can be seen in Figure 8b, the seasonal pattern of group B at Hart is similar to the trend observed for SWMREC. Although the highest rate was not obtained at the first measurement on July 28, the rate, 24 $\mu mol \cdot m^{-2} \cdot s^{-1}$, was slightly lower than the highest rate, 25.7 $\mu mol \cdot m^{-2} \cdot s^{-1,}$ obtained at the second measurement on August 7. There was a large decrease in photosynthetic rate during the third measurement (Aug. 11). The rate dropped from the previous measurement of 25.7 to 19.9 $\mu mol \cdot m^{-2} \cdot s^{-1}$. It is speculated to be due to the influence of dramatic changes of weather on that date. The weather changed from sunny to partly cloudy to cloudy that day. '86Ram-3' and '86Sam-3' were measured under sunny condition, the PAR values were 1680, and 1740 $\mu mol \cdot m^{-2} \cdot s^{-1}$

Figure 8. Seasonal changes of asparagus photosynthetic rate at Hart.
Assimilation rate for each date of group A was the average of 'Jersey Giant', 'UC86-11', '44Px22-8', and 'Tainan-3'.
Assimilation rate for each date of group B was the average of '86Ram-3', '86Sam-3', 'Hart-3', and 'Franklim'.
Seven measurements in group A were conducted on: 07/27, 08/06, 08/10, 08/21, 08/22, 09/04, 09/16.
Seven measurements in group B were conducted on: 07/28, 08/07, 08/11, 08/22, 09/03, 09/04, 09/16.





for these two measurements respectively. It changed to partly cloudy when measuring 'Hart-3', with a PAR value of 907 μmol · m^{-2} · s^{-1}. It was very cloudy (PAR, 487 μmol · m^{-2} · s^{-1}) during the measurement of 'Franklim'. In a study on asparagus photosynthesis, Sawada et al., (1965), found that asparagus assimilation rate was correlated closely with the prevalent weather conditions. The low average photosynthetic rate on Aug. 11 was very likely due to the changing of weather conditions during that day's measurements. The photosynthetic rate was decreasing after Aug., 22 as shown in Figure 8b. It reached the lowest point of the season at the last measurement (Sept. 16) with a rate of 14.7 μmol · m^{-2} · s^{-1}. The above pattern shows that the highest rate of photosynthetic rate for group B at Hart occurred in early August. From July to August, asparagus plants engaged in active photosynthesis with high photosynthetic rates. Upon entering September, photosynthetic rates began to decrease.

The pattern of group A (Figure 8a) is different from that of group B. The rates in the first three measurements, 20.9 μmol · m^{-2} · s^{-1} on July 27, 22.3 on Aug. 6, and 22.2 on Aug. 10 were close to each other, but they were lower than the highest rate of the season obtained at Aug. 21 (27.3 μmol · m^{-2} · s^{-1}). In this case, the peak photosynthetic rate of the season was not at the end of July or early August, but was reached near the end of August. After reaching this peak level, photosynthetic rate decreased during subsequent measurements. It reached the lowest point also at the last measurement of the season. The latter part was similar to that observed in the other three groups. The reason for this difference of seasonal photosynthetic rate change pattern is not understood. This discrepancy might result from the influences of factors such as weather conditions and human error. The first two measurements were done under partly sunny day; the third measurement was made on a cloudy day.

Despite the differences of seasonal photosynthetic rate change patterns among different groups, there is good reason to describe the asparagus seasonal photosynthetic rate change as such: the highest photosynthetic rate of the season was reached at the end

of July or early August. During this time, asparagus photosynthesizes very actively at a high rate in response to the high demand of depleted sinks. During August, photosynthetic rate is high and maintains a relatively steady level. During September, photosynthetic rate decreased greatly. In all four groups, the lowest rate of the season was found at the last measurement. Ferns began to turn yellow starting in mid to late September, which marked the end of the growing season. Feedback mechanisms and unfavorable weather conditions cause the decrease of photosynthetic rate in September.

Part Three: Photosynthetic rate variability of eight asparagus genotypes and its correlation with yield and specific leaf weight.

One of the main goals in this study of asparagus photosynthesis was to investigate the variability of photosynthetic rate. Photosynthetic rates of the eight asparagus genotypes obtained from field measurements are shown in Figure 9. It appears that there is great variation among different genotypes. 'Franklim' lead the genotypes with an average photosynthetic rate of 27.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$. 'Hart-3', 'Jersey Giant', '44Px22-8' and 'UC86-11' exhibited similar photosynthetic rates. 'Tainan No.3', '86Ram-3', and '86Sam-3' exhibited relatively low photosynthetic rates. Each of the eight genotypes showed great within-genotype variation in photosynthetic rate. This reflects the fact that each genotype was measured at different time of the day, under different weather conditions, and measured at different dates throughout the season.

Analysis of variance (ANOVA) was conducted on photosynthetic rate data. Table 9 is the ANOVA table. The experiment adopted a split-plot design. The main plots were eight genotypes; the sub-plots were seven dates of measurements.

The upper part of this ANOVA table is the analysis of the main plot, the genotypic variation in photosynthetic rate. Two locations were used as two blocks in this experiment. The F value for genotypic effect (2.93) indicates that these eight asparagus genotypes are significantly different in their photosynthetic ability ($Pr<0.1$). For this preliminary investigation on asparagus genotypic variability a broader standard ($Pr<0.1$) is used, as it is suitable in some preliminary studies.

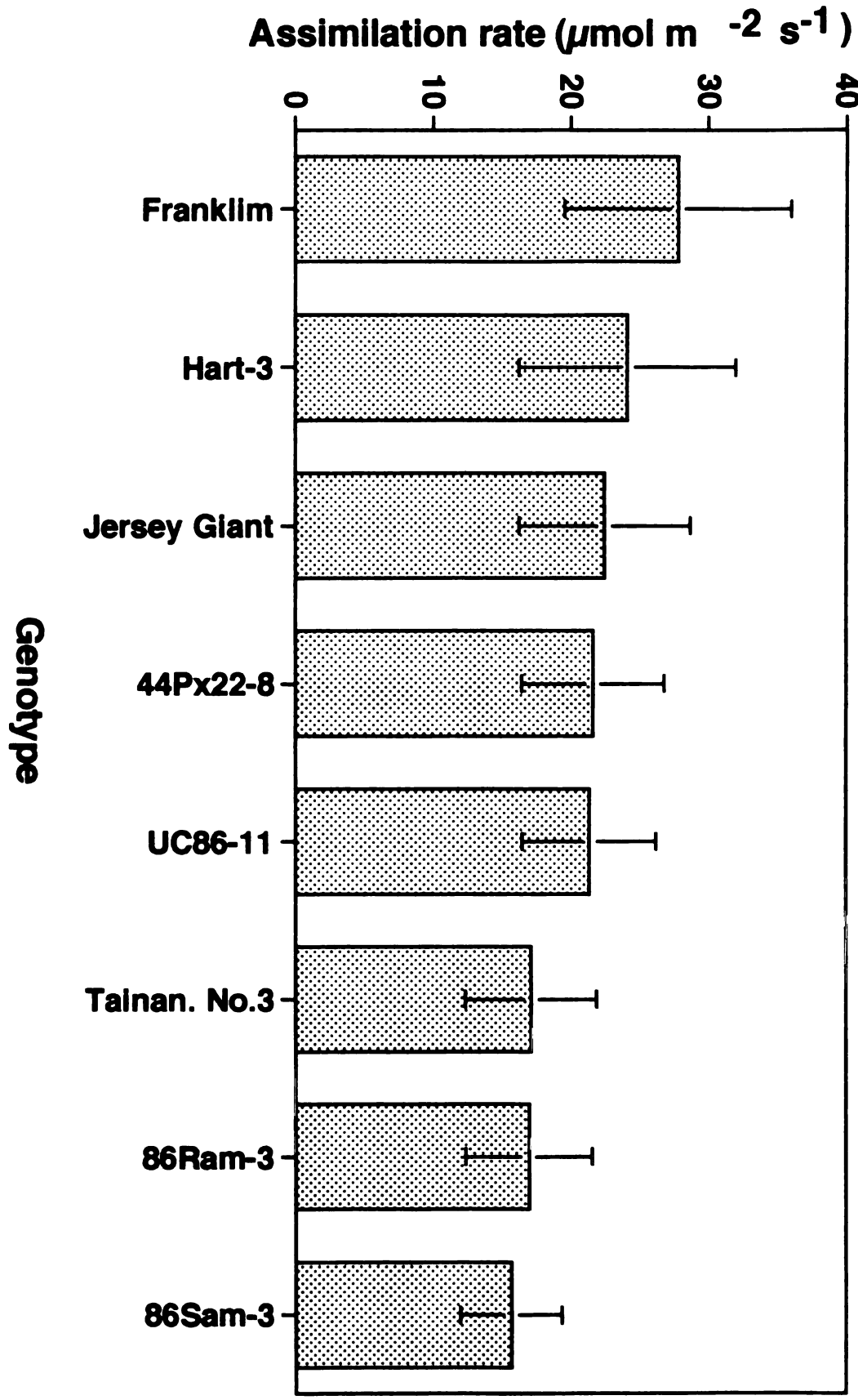


Figure 9. Photosynthetic rates of elght asparagus genotypes. Value for each genotype is the average of 14 measurements. Standard deviation of each genotype is shown.

Table 9. Analysis of Variance of Photosynthetic Rate Data.

Source	DF	Sum of Squares	Mean Squares	F value
Total for location	1			
Total for cultivar	15	2266.3		
location(block)	1	53.6		
cultivar	7	1649.9	235.7	2.932*
Error a (block.*cult.)	7	562.8	80.4	
Total of assimilation rate	111	5247.9		
total for cultivar	15	2266.3		
date of measurement	6	1380.6	230.1	10.7**
date*cultivar	42	571.6	13.6	0.634
Error b (date*cult.*block+date*block)	48	1029.4	21.5	

* F value significant at 10% level.

** F value significant at 1% level.

The lower part of the ANOVA table are analyses on the sub-plot, the date effect, and the interaction between genotype and measuring date. The date factor is responsible for a large portion of the total variation. Discussions on seasonal changes of photosynthetic rate in part two of this chapter showed that asparagus photosynthetic rate changed greatly during the season. The F value for this factor, 10.7, shows that there was a highly significant difference in photosynthetic rate among the seven measurement dates. ($Pr<0.01$). This verified the conclusion given in the previous section that asparagus photosynthetic rate changed significantly over the season from July to September. The F value for the interaction of measuring date and genotype is not significant; this indicates that there were no significant differences in the patterns of seasonal photosynthetic rate change among the eight genotypes.

A multiple comparison test, Tukey's Studentized Range (HSD) Test, was conducted to look at the photosynthetic rate differences of the eight asparagus genotypes . Table 10 shows the grouping of these eight genotypes with respect to photosynthetic rate.

The results of Tukey's test indicate that photosynthetic rate of 'Franklim' was significantly higher than all except 'Hart-3' and 'Jersey Giant'. 'Hart-3', 'Jersey Giant', '44Px22-8', and 'UC86-11' had relatively higher photosynthetic rates, but had no significant differences with each other. 'Tainan No. 3', '86Ram-3', and '86Sam-3' were in one group in the lower range. '86Sam-3' had significantly lower photosynthetic rate than all except '86Ram-3' and 'Tainan No.3'.

Table 10. Photosynthetic rate differences of eight asparagus genotypes. (HSD).

Ranking	Genotype	Mean Pn Rate+	Tukey	Grouping*
1	Franklim	27.789	A	
2	Hart-3	24.096	A	B
3	Jersey Giant	22.446	A	B
4	44Px22-8	21.6	C	B
5	UC86-11	21.343	C	B
6	Tainan No.3	17.129	C	D
7	86Ram-3	16.967	C	D
8	86Sam-3	15.668		D
HSD		5.55	Pr<0.05	

+: Average of 14 measurements; the unit is $\mu mol \cdot m^{-2} \cdot s^{-1}$.

*: Cultivars sharing the same letter are not significantly different.

One of the main interests of this study was to see if asparagus' photosynthetic ability has any correlation with yield performance. Yield data for these eight genotypes are available from four multi-year yield trial experiments; hybrid trials at Hart and SWMREC and clone trials at these same locations. There are great differences in the yield performances of the eight genotypes. 'Hart-3' has the highest average yield, 4934 lb/acre, which is about four times more than the lowest yielding genotype, '86Sam-3' (1220 lb/acre). The four top-yielding genotypes in order are 'Hart-3', '44Px22-8', 'Franklim', and 'Jersey Giant'. Coefficient of correlation between photosynthetic rate and yield was calculated. A positive coefficient of correlation, 0.786 was found. It is significant at the 5% level. This indicates that high-yielding genotypes tend to have high photosynthetic rates.

'Franklim' is an all-male hybrid introduced from Netherlands, it ranks first in the yield trial at Hart, leading the 34 cultivars, and ranks 8th at SWMREC. Different from the other three high-yielding cultivars, 'Franklim' has a short, concentrated plant architecture. The high yield performance and small above-ground plant architecture indicates strongly that 'Franklim' has a high carbohydrate distribution ratio favoring the underground sinks, which implies that it has a high harvet index. 'Hart-3', an all-male M.S.U. clone, is the highest-yielding genotype; it ranks first in M.S.U. clone trial at Hart and second at SWMREC. Its photosynthetic rate is the second highest among the eight genotypes. 'Hart-3' is tall and has a very vigorous fern growth. 'Hart-3' has a very good likelihood to develop into a successful cultivar for commercial production. 'Jersey Giant' is a well-established commercial cultivar. It ranks second in the cultivar yield trial at SWMREC and 7th at Hart. Its photosynthetic rate ranks third among the eight asparagus genotypes. '44Px22-8' is an all-male hybrid line developed in New Jersey. It has a similar plant architecture as 'Hart-3', the two tallest genotypes. '44Px22-8' ranks first in yield trial at SWMREC and third at Hart. These four cultivars all have outstanding yield performance and are superior in photosynthetic ability. This indicates

the possibility that high photosynthetic rate is one of the factors that results in genotypes with superior yield performance.

Genotypes with high photosynthetic rate can be good breeding resources for improving asparagus as a crop. There was evidence that CO_2 exchange rate is a trait of high heritability. (Crosbie et al., 1978; Vietor et al., 1979; Wilson et al., 1969). Thus, one possible means of using this resource is to use high photosynthetic rate genotypes, such as 'Franklim', as parental materials in asparagus breeding. Improvements in crop yield have been realized largely by increasing harvest index, which is the ratio of economic sink to total plant weight, but clearly there is a limit to how far this can go. Improvement in a plant's photosynthetic rate may become essential to further increases in yield. (Gifford and Evans, 1981).

'Tainan No.3', '86Ram-3', and '86Sam-3' have low photosynthetic rates comparing to others. 'Tainan No.3' has poor yield performance in the yield trial at both locations. Interestingly, 'Tainan No.1', and 'Tainan No. 2', the other two cultivars introduced from sub-tropical Taiwan, have very similar yield performances as 'Tainan No.3'. They are among the lowest yielding cultivars in the yield trial. The similar yield performances of the three sub-tropical cultivars indicate that they might suffer from adapting to the growth conditions at Michigan. '86Sam-3' has the poorest yield performance in the yield trials at these two locations. Low photosynthetic rate might be one of the reasons for the low yield performance.

A morphological character, specific leaf weight (SLW=leaf dry weight per unit area), often is found to correlate with photosynthetic rate. (Barnes et al., 1969; Dornhoff et al., 1970; Augustine et al., 1979). Leaf area of asparagus was measured involving three replications for each genotype. Leaf area ratio (LAR) and specific leaf weight (SLW) of each genotype were calculated. (Table 13). Interestingly, the highest photosynthetic rate genotype, 'Franklim', had the highest specific leaf weight (13.55 mg cm^{-2}) and the lowest photosynthetic rate genotype, '86Sam-3', had the lowest specific leaf

weight (10.43 mg $\cdot$ cm^{-2}). Analysis of Variance (ANOVA) is used to test the significance of differences of specific leaf weight of the eight genotypes. An F value of 6.01 indicates significant differences at the 5% level. Least significant difference (LSD) test was conducted to compare specific leaf weights of the eight genotypes. (Table 11). ANOVA indicates that significant differences in SLW exist among asparagus genotypes. LSD indicates that SLW of 'Franklim' is significantly higher than all others except 'Jersey Giant' and '44Px22-8'; these two genotypes were not significantly different from 'UC86-11', '86Ram-3', 'Tainan No.3', and 'Hart-3'. All the above seven genotypes are significantly higher than '86Sam-3' in SLW.

The coefficient of correlation (r) between specific leaf weight and photosynthetic rate was calculated. A significant positive correlation, 0.74, was found ($Pr<0.05$). This is in accordance with a similar finding by previous studies with other species that high photosynthetic rate often is correlated with high specific leaf weight (Barnes et al., 1969; Dornhoff et al., 1970; Augustine et al., 1979). The correlation between SLW and photosynthetic rate is plotted (Figure 10) to show the linearity between these two parameters. For high-SLW asparagus genotypes, there is more total weight in a unit fern area basis, indicating that there are more cells involved in photosynthesis, and at a unit time such plants can fix more CO_2. The difference in SLW among asparagus genotypes is likely one important causal factor responsible for the significant differences of

Figure 10. Correlation between photosynthetic rate and specific leaf weight.

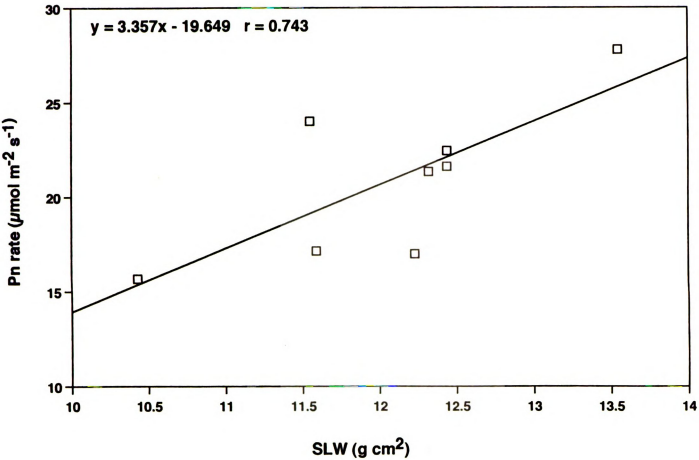


Table 11. Leaf area (LA), specific leaf weight (SLW) and leaf area ratio (LAR) of eight asparagus genotypes. There were three replications for leaf area measurement.

Genotypes	LA (cm^2)	SLW ($mg\ cm^{-2}$))	Average	LAR ($cm^2\ g^{-1}$)	Average
Franklim	338.6	13.73		72.817	
	383	13.39		74.659	
	495	13.52	13.55A	73.991	73.82
Jersey Giant	368.2	12.22		81.822	
	449	11.92		83.925	
	458.8	13.19	12.44AB	75.835	80.53
44Px22-8	329	11.95		83.715	
	412.8	12.48		80.155	
	315.7	12.89	12.44AB	77.568	80.48
UC86-11	336.6	13.34		74.967	
	401.2	11.54		86.652	
	386.4	12.09	12.32B	82.741	81.45
86Ram3	364.1	13.46		74.306	
	444	11.91		83.932	
	438.8	11.13	12.23B	88.468	82.24
TaiNan-3	277.5	11.14		89.806	
	307.6	11.57		86.404	
	505.2	12.05	11.59B	82.956	86.39
Hart-3	393.6	11.51		86.887	
	442.8	11.54		86.654	
	412.4	11.59	11.55B	86.276	86.61
86Sam3	370	10.81		92.500	
	438.4	9.83		101.717	
	464.4	10.66	10.43C	93.818	96.01
LSD			1.1		

LSD: Least significant difference test ($Pr<0.05$)
Genotypes sharing the same letter are not significant at 5% level.

photosynthetic rate among genotypes. Specific leaf weight is a morphological character that is easier to measure than photosynthetic rate. When interests arise to select genotypes with high photosynthetic rate, we can instead select high SLW genotypes. This could reduce the amount of work involved.

Part Four: Chlorophyll and protein content of asparagus fern.

Chlorophyll content of asparagus fern was reported by Downton et al., (1975) to be 1.35±0.02 (3) mg · g fresh wt^{-1}. Chlorophyll content of the eight genotypes was measured. Table 12 gives the chlorophyll contents of the eight genotypes.

The chlorophyll content of the genotype used in the study of Downton (1975) was much lower that the chlorophyll content of the genotypes of this study; it is lower than the lowest genotype ('86Ram-3'). Analysis of variance was conducted to test the significance of differences in total chlorophyll content of the eight genotypes. Differences in total chlorophyll contents of the eight asparagus genotypes are highly significant. ($F=6.88$; $Pr<0.01$). This indicates that there is a wide variation of chlorophyll content among different asparagus genotypes. It was observed in the field that some genotypes, '44Px22-8', and 'Franklim', had dark green ferns, while the color of some genotypes, 'Tainan No.3', '86Ram-3', were not as green as others. Least significant difference (LSD) test was performed to compare chlorophyll content of the eight genotypes. '4Px22-8' had the highest chlorophyll content (1.79 mg · g $f.wt.^{-1}$), significantly higher than all except 'Franklim' and 'UC86-11'. These two are significantly higher than the others except 'Jersey Giant'. At the low end, '86Ram-3' is significantly lower in chlorophyll content than the other seven genotypes. (1.37 mg · g $f.wt.^{-1}$).

The ratios of chlorophyll a and chlorophyll b of the eight genotypes were calculated (Table 12), the range of this ratio is from 3.00 to 3.32. A ratio about three is expected for green photosynthesizing tissues.

The coefficient of correlation between photosynthetic rate and chlorophyll content was calculated ($r=0.509$); this is not a significant correlation. This indicates that there might not be any positive correlation between photosynthetic rate and chlorophyll content in asparagus. However, with a larger sample size a significant relationship between them might be revealed. The high variability of asparagus chlorophyll content is

Table 12. The chlorophyll contents of eight asparagus genotypes.
Each value is the average of five samples.
Standard deviation of each measurement is given.

Genotypes	Chlorophyll a mg.g f.wt.$^{-1}$	Chlorophyll b mg.g f.wt.$^{-1}$	Chla/Chlb	Total Chlorophyll mg.g f.wt.$^{-1}$	
44Px22-8	1.36±0.077	0.43±0.018	3.16	1.79±0.093	A
Franklim	1.32±0.076	0.44±0.025	3.00	1.76±0.1	AB
UC86-11	1.26±0.105	0.41±0.026	3.07	1.67±0.13	AB
Jersey Giant	1.23±0.037	0.41±0.012	3.00	1.64±0.043	BC
86Sam-3	1.23±0.1	0.37±0.044	3.32	1.62±0.143	C
TaiNan-3	1.21±0.092	0.39±0.026	3.10	1.6±0.116	C
Hart-3	1.18±0.124	0.38±0.032	3.11	1.57±0.155	C
86Ram-3	1.04±0.042	0.33±0.021	3.15	1.37±0.062	D
LSD:				0.1435	

LSD: least significant difference (Pr<0.01).

Genotypes sharing the same letter are not significantly different.

an indication of the genotypic diversity of asparagus as a result of human breeding efforts. Because of the varied sources of the eight genotypes, Netherlands, Taiwan, California, New Jersey, Michigan, the variability in chlorophyll content reflects that this diversity of regions may influence chlorophyll content during the development of these genotypes.

Whole protein content of asparagus ferns was studied on three genotypes. 'Franklim', 'Jersey Giant', and 'Tainan No.3', using the Bradford method. Six samples were used for each protein determination of each cultivar. Table 13 gives the protein contents of the three genotypes. ANOVA test shows that there is no significant difference (F=1.5) among the three genotypes in their whole protein content.

Table 13 Whole protein content of three asparagus genotypes. Six samples were conducted for each genotypes, standared deviation is shown.

Genotypes	Protein content mg g fwt^{-1}	Average
Franklim	17.02	
	13.38	
	14.81	
	12.58	
	12.33	
	16.4	14.42±1.98
Tainan No.3	12.08	
	11.7	
	16.29	
	14.59	
	13.39	
	18.6	14.44±2.64
Jersey Giant	10.95	
	10.64	
	14.06	
	10.79	
	14.9	
	13.74	12.51±1.92

Part Five: Rubisco activity of three asparagus genotypes.

Ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco) is the most abundant leaf protein in green plants; it plays a key role in carbon assimilation. Rubisco catalyzes the irreversible carboxylatoin of RuBP to form two molecules of 3-phosphoglycerate, which is the first step of CO_2 fixation in the dark reaction in C_3 plants.

To optimize the method for studying asparagus rubisco activity, we tested the effects of enzyme activation and sample desalting; we determined the optimum concentration of RuBP and optimum amount of enzyme solution to use in the asparagus rubisco reaction. We studied also the linearity of CO_2 fixation by asparagus rubsico.

Studies on rubisco activity show that the activation process can enhance rubisco activity of some species. (Lorimer et al., 1977; Laing et al., 1976). Kinetic analyses indicated that rubisco is activated in three steps according to the equation:

$$E^*(\text{inactive}) \cdots\cdots E + CO_2 \cdots\cdots E\text{-}CO_2 + Mg^{++} \cdots\cdots E\text{-}CO_2\text{-}Mg^{++}(\text{active})$$

Asparagus rubisco was activated by preincubation with CO_2 and Mg^{++} for five min before adding RuBP to initialize the reaction. Figure 11 shows the comparison of rubisco activity between the activation treatment and control. The activation process did not show any enhancing effect on asparagus rubisco activity; instead, it caused a decrease of rubisco activity. From the results it can be concluded that activation is not a necessary step for asparagus rubisco study.

Desalting of purified asparagus rubisco was performed according to the method of Neal and Florini (1973). It was stated that desalting can remove low-molecular-weight compounds which might interfere with the assay. Figure 12 shows that the desalting treatment did not increase asparagus rubsico activity. The rubisco activity of the control is slightly higher than for the desalting treatment. Desalting had no positive effect on increasing asparagus rubisco activity and hence is not a necessary step in studying asparagus rubisco. Mg^{++} and HCO_3^- were added in the desalting buffer, because their

Figure 11. Time courses of CO_2 fixation showing the difference between control and activation treatment.

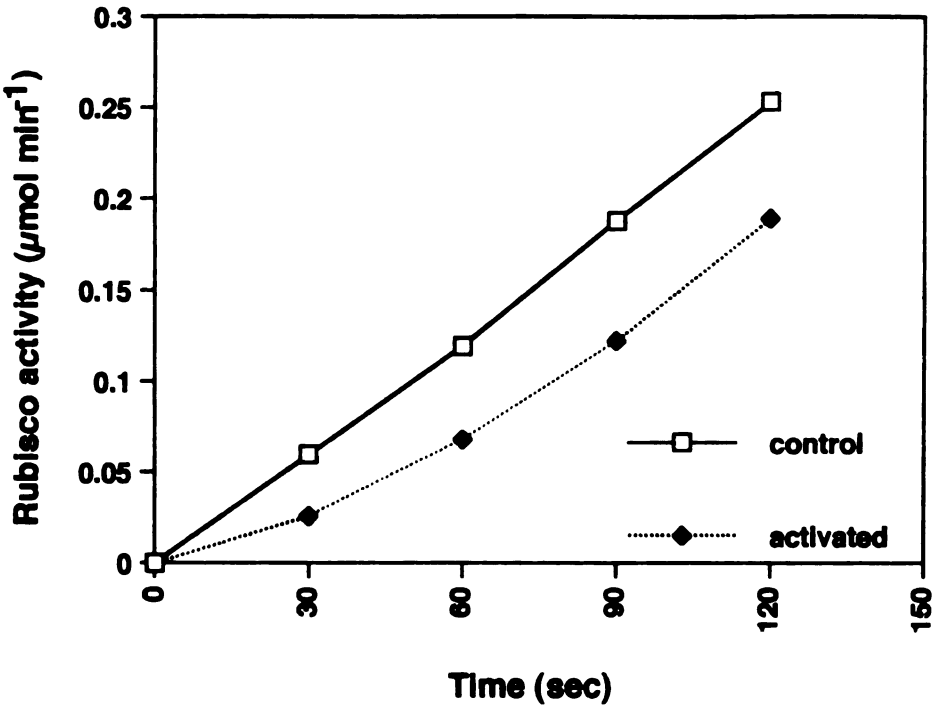
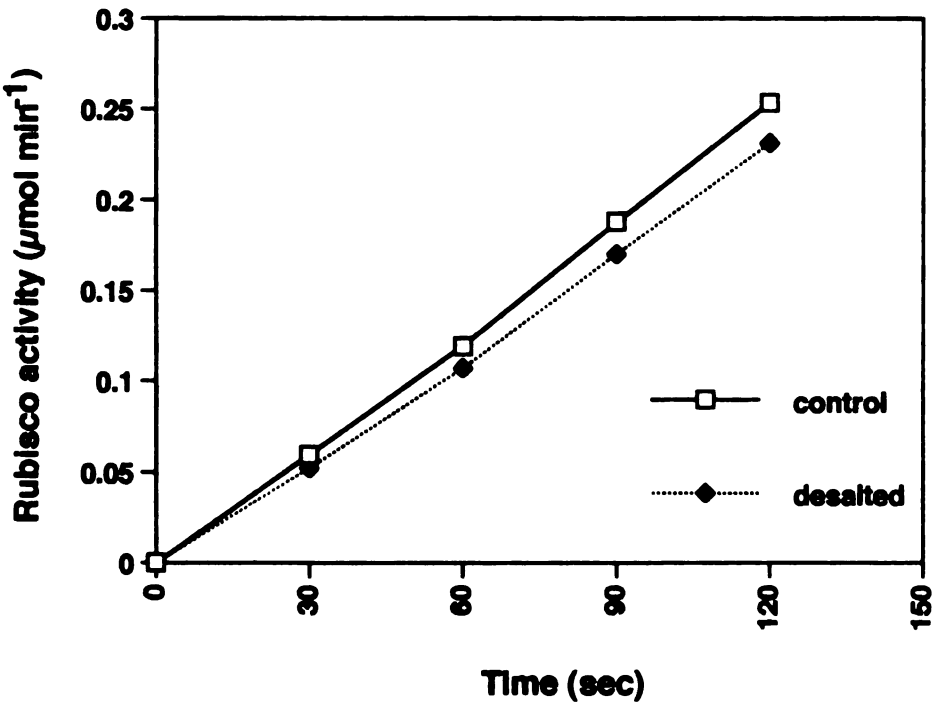


Figure 12. Time courses of CO_2 fixation showing the difference between control and desalting treatment.



presence can activate the enzyme and such activation process might increase rubisco activity. Like the activation treatment prior to reaction process, such activation during desalting had no evident positive effect on asparagus rubisco activity.

Three different amounts of purified rubisco solution, 25 , 50 , and 75 μl, were compared to determine the optimum amount of enzyme solution for maximization of the reaction. Figure 13 gives the result of this comparison. As can be seen in Figure 13, 50 μl enzyme solution gives the maximum activity of asparagus rubisco.

Four concentrations of RuBP, 0.25 , 0.5, 1, and 2 mM were compared to determine the optimum concentration for asparagus rubisco acitivity. Table 14 shows the comparison among these four concentrations. Rubisco activity was lowest at 0.25 mM RuBP, indicating this concentration is not high enough for maximum reaction. Rubisco activity was highest at a concentration of 1 mM. This is presented as the optimum concentration for asparagus rubisco study.

As shown in Figure 11, 12, and 13 a good linearity of asparagus rubisco activity is found in this study. A linearity permits an accurate estimation of asparagus rubisco activity without doing a time-course study, but stopping the reaction at 120 seconds, which was the protocol adopted in following assays.

After establishing the optimum reaction conditions for asparagus rubisco assay, rubisco activities of the three genotypes were investigated. Twelve replications were used in each genotype assay. Table 15 shows the result of rubisco activities of the three asparagus genotypes. Rubisco activity is expressed in two different units: μmol CO_2 mg chl^{-1} min^{-1}, μmol CO_2 mg $protein^{-1}$ min^{-1}. Compared with the asparagus rubisco activity reported by Downton (1975), which was 10.7±1.16 μmol CO_2 mg chl^{-1} min^{-1} using three replications, all the values we obtained are lower.

Figure 13. Time courses of CO2 fixation of three different amount of purified enzyme.

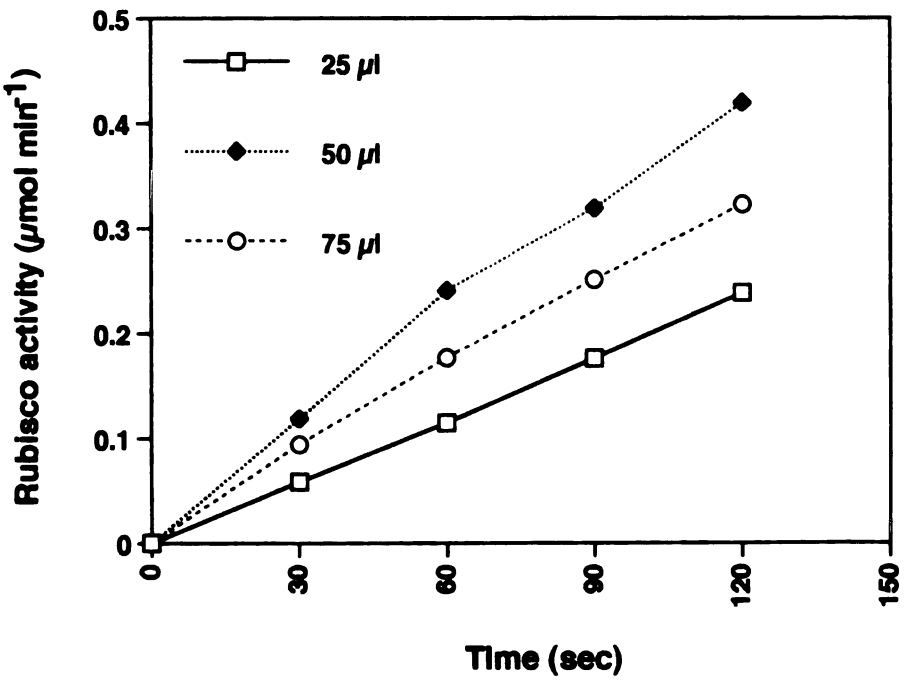


Table 14. Asparagus rubisco activity at different concentrations of RuBP.

Three replications for each RuBP concentration.

Concentration of RuBP	Rubisco activity (μmol min-1)	Average
0.25 mM	0.0479	
	0.05	
	0.0512	0.0497
0.5 mM	0.1003	
	0.0996	
	0.1004	0.1001
1 mM	0.1187	
	0.1179	
	0.1137	0.1168
2 mM	0.1103	
	0.0983	
	0.1042	0.1043

Table 15 Rubisco activities of three asparagus genotypes. For each genotype, 12 replications were used.

Genotype	Rubisco activity ($\mu mol\ mg^{-1}$ chl min^{-1})	Average	Rubisco activity ($\mu mol\ mg^{-1}$ protein min^{-1})	Average
Franklim	3.39		0.467	
	3.055		0.4	
	4.725		0.427	
	3.456		0.661	
	3.037		0.484	
	4.698		0.467	
	8.475		0.632	
	7.935		0.592	
	6.427		0.62	
	6.398		0.617	
	7.65		0.641	
	6.118	5.447 A	0.512	0.543A
Tainan No.3	4.149		0.475	
	4.48		0.513	
	4.258		0.442	
	4.618		0.531	
	3.596		0.576	
	5.137		0.449	
	4.075		0.46	
	3.959		0.447	
	4.271		0.453	
	3.891		0.412	
	4.782		0.392	
	4.788	4.334 B	0.425	0.465AB
Jersey Giant	3.515		0.534	
	3.441		0.523	
	3.899		0.593	
	3.309		0.351	
	3.652		0.425	
	3.197		0.394	
	5.158		0.636	
	3.16		0.431	
	3.031		0.413	
	3.499		0.361	
	5.479		0.565	
	3.874	3.768 B	0.486	0.476B
LSD		1.04		0.068

LSD: least significant difference. $Pr<0.05$.

For 'Franklim', rubisco activity was 5.447±1.98 μmol $CO_2 \cdot mg^{-1} \cdot chl \cdot min^{-1}$. For 'Tainan No.3', it was 4.334±0.44 μmol $CO_2 \cdot mg^{-1} \cdot chl \cdot min^{-1}$. For 'Jersey Giant', the activity was 3.768±0.77 μmol $CO_2 \cdot mg^{-1} \cdot chl \cdot min^{-1}$. The low rubisco activity in this study compared to the previous study might be caused by: (i) genotypic differences; the genotype used in their study could have high rubisco activity, (ii) different time of sampling, (according to Fleck and Marco (1981), rubisco activity is different at different times in the season), (iii) our samples were stored in a -80°C freezer during the two-month lab study, which might cause the loss of rubisco activity, and (iv) experimental errors.

ANOVA was conducted to test the significance of differences among these three genotypes. LSD was calculated and comparisons are shown in Table 15. In both methods of rubisco activity expression, one based on chlorophyll (hence referred as chlorophyll expression) and one based on protein (hence referred as protein expression), F values, 5.55 for chlorophyll expression, and 3.23 for protein expression, indicate that rubisco activities of the three asparagus genotypes are significantly different. ($Pr<0.05$). This is an indication that there is significant difference in rubisco activity among asparagus genotypes. For chlorophyll expression, LSD test indicates that 'Franklim' had significantly higher rubisco activity than 'Tainan No.3', and 'Jersey Giant', and these two are not significantly different from one another. For protein expression, 'Franklim' had significantly higher rubisco activity than 'Tainan No.3', but not significantly higher than 'Jersey Giant'. 'Jersey Giant' was not significantly higher than 'Tainan No.3'.

The values obtained for rubisco activity and photosynthetic rate should be in a similar range when they are expressed in the same unit. Photosynthetic rate expression of the three asparagus genotypes were converted from μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ to μmol $CO_2 \cdot mg^{-1}$ chl $\cdot min^{-1}$, the same as the expression of rubisco activity.

Table 16. Relationship between rubisco activity and photosynthetic rate.

Genotype	Rubisco activity* ($\mu mol \cdot mg^{-1} \cdot chl \cdot min^{-1}$)	Photosynthetic rate** ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Photosynthetic rate ($\mu mol \cdot mg^{-1} \cdot chl \cdot min^{-1}$)
Franklim	5.447	27.79	6.994
Tainan No.3	4.334	17.13	5.549
Jersey Giant	3.768	22.45	6.614

* Average of 12 replications. ** Average of 14 replications.

The closeness between the values of rubisco activity and photosynthetic rate, when expressed in the same unit, verified the acceptability and accuracy of the photosynthetic rate data obtained from field measurements.

Correlation between rubisco activity and photosynthetic rate of asparagus can not be studied due to the small number of subjects involved. These data do not indicate that higher rubisco activity is necessarily associated with high photosynthetic rate. Although the genotype with significantly higher rubisco activity, 'Franklim', is also the one with the highest photosynthetic rate; the same relationship does not hold in the comparison of 'Jersey Giant', and 'Tainan No. 3', where they are not significantly different in rubisco activity, but are significantly different in photosynthetic rate. The other difficulty in correlating rubisco activities obtained in lab study with photosynthetic rates measured in field, is that optimum reaction conditions, such as CO_2 concentration, are supplied for rubisco reaction in a lab study, but under field conditions rubisco works under the influences of many other variables.

In this study, optimum reaction conditions for studying asparagus rubisco were established. Activation and desalting treatments do not enhance asparagus rubisco activity. Optimum concentration of RuBP is 1 mM, and optimum amount of purified enzyme solution is 50 μl for asparagus rubisco activity. There are significant differences among the three asparagus genotypes in their rubisco activity. 'Franklim' has a significantly higher rubisco activity among these three genotypes. High rubisco activity might be a factor for high photosynthetic rate, but the relationship between asparagus photosynthetic rate and rubisco activity needs further studies for elucidation.

Chapter four: Conclusions

The pattern of daily photosynthetic rate changes of asparagus plants was studied. The results indicated that there was not a universal daily photosynthetic rate change pattern that can be applied to di`fferent locations, different times in a season, different environmental conditions, or different cultivars. Daily asparagus photosynthetic rate changes, on sunny days during the active growth period, from July to August, as follows: photosynthetic rate begins to increase after sunrise with increasing temperature and PAR in the presence of high environmental CO_2 concentration; this increase reaches a maximum between 9 AM and 10 AM, followed by a period of photosynthesis at a high rate for a few hours to about 1:00 PM; the peak of photosynthetic rate during the day is within this period at anywhere from 11:30 AM to 1 PM. Then the photosynthetic rate drops to a lower level; this decrease is primarily due to a reduction of stomatal conductance combined with other factors such as decreasing environmental CO_2 concentration, increasing temperature, and possibly light inhibition. Photosynthesis is carried out at this lower rate during most of the afternoon and decreases to the lowest daylight point after 5:00 PM. It was found that a very prominent factor limiting photosynthetic rate is CO_2 diffusion resistance, especially stomatal conductance (g_s). Data on the changes of stomatal conductance and intercellular CO_2 concentration make it clear that these factors play a direct role in the changes of asparagus photosynthetic rate.

The pattern of seasonal photosynthetic activity changes were established by measuring photosynthetic rates through the season from the end of July to the end of September. The highest photosynthetic rate of the season was reached between the end of July and early August. During this time, asparagus photosynthesizes very actively at a high rate in response to the high demand of depleted sinks. During August, photosynthetic rate is high and maintains a relatively steady level. During September,

photosynthetic rate decreased greatly. The lowest rate of the season was found at the end of September. Feedback mechanisms and unfavorable weather conditions cause the decrease of photosynthetic rate in September.

The eight asparagus genotypes employed in this experiment are significantly different in their photosynthetic ability ($Pr<0.1$). Multiple comparison method (Tukey's test) shows that 'Franklim' had a significantly higher photosynthetic rate than the other genotypes; 'Hart-3', 'Jersey Giant', '44Px22-8', and 'UC86-11' had relatively high photosynthetic rates, but there were no significant differences among them; 'Tainan No.3', '86Ram-3', and '86Sam-3' exhibited relatively low photosynthetic rates; the rate of '86Sam-3' was significantly lower than most other genotypes.

The coefficient of correlation between photosynthetic rate and yield (0.786) was significant ($Pr<0.05$). This indicates that high-yielding genotypes tend to have high photosynthetic rates. Four genotypes with outstanding yield performances were also superior in photosynthetic ability. This indicates the possibility that high photosynthetic rate is one of the factors that contributes to superior yield performance. Genotypes with high photosynthetic rate can be good breeding resources for improving asparagus as a crop. Significant differences exist among the eight asparagus genotypes in specific leaf weight ($Pr<0.05$). The coefficient of correlation between specific leaf weight and photosynthetic rate (0.74) was significant ($Pr<0.05$). This is in accordance with a similar finding in previous studies with other species, that high photosynthetic rate often is correlated with high specific leaf weight. The differences in SLW among asparagus genotypes is likely one important causal factor responsible for the significant differences of photosynthetic rates among genotypes. Specific leaf weight can be used as a selection criterion for selecting high photosynthetic rate genotypes.

Chlorophyll contents of the eight asparagus genotypes were determined. Differences of total chlorophyll content of the eight asparagus genotypes are highly significant. ($F=6.88$; $Pr<0.01$). The ratio of chlorophyll a and chlorophyll b in asparagus

fern is about 3. The coefficient of correlation between photosynthetic rate and chlorophyll content (0.509) was not significant.

The method for the assay of asparagus rubisco was optimized, and rubisco activities of three asparagus genotypes were measured. It was found that activation and desalting treatments do not enhance asparagus rubisco activity. Optimum concentration of RuBP is 1 mM, and the optimum amount of purified enzyme solution is 50 μl for asparagus rubisco activity. Asparagus rubisco activity has a good linearity with time. There are significant differences among the three asparagus genotypes in their rubisco activity ($Pr<0.05$). 'Franklim' had a significantly higher rubisco activity among these three genotypes. High rubisco activity might be a factor for high photosynthetic rate, but the relationship between asparagus photosynthetic rate and rubisco activity needs further study.

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