

THESIS

SOIL AND PLANT FACTORS ASSOCIATED WITH
IRON CHLOROSIS OF KENTUCKY BLUEGRASS

Submitted by

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED
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ABSTRACT

SOIL AND PLANT FACTORS ASSOCIATED WITH IRON CHLOROSIS OF KENTUCKY BLUEGRASS

Iron (Fe) chlorosis is a common problem on turfgrasses grown under alkaline soil conditions. The objective of this study was to determine the iron uptake efficiency of different varieties of Kentucky bluegrass. In this study, 25 varieties and 5 blends of Kentucky bluegrass (Poa pratensis L.) were utilized. Maintenance practices for all varieties were the same, and at the time of sampling the grasses were three years old, were well established, and had not been treated with iron containing materials. Soil pH, soil available iron, plant total iron, plant chlorophyll content, and plant iron uptake efficiency were determined. Also, the visual appearance of the turf was scored before sampling. Statistical treatment indicated significant differences in the total iron and chlorophyll content of the varieties. The following significant correlations were observed: (1) soil available iron to iron uptake efficiency (negative); (2) plant total iron to iron uptake

efficiency (positive); (3) plant chlorophyll content to plant iron content (positive); (4) plant chlorophyll content to iron uptake efficiency (positive). The varieties and blends were significantly different in their iron uptake efficiency.

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INTRODUCTION

An effective turfgrass maintenance program is dependent upon adequate fertilization to provide essential nutrients for optimum plant growth and appearance. Failure to produce an acceptable green turfgrass color because of plant chlorosis can result from several nutritional deficiencies or excesses. Under alkaline soil conditions, low soil available iron is a primary cause of chlorosis. The function that iron plays in turf maintenance is essentially that of providing a green, aesthetically acceptable turf.

In many parts of the world, available soil iron is too low to support the production of an acceptable turf. Available iron in soil is a factor related to iron chlorosis, but it can not be used as an absolute index to the occurrence of iron chlorosis (5).

Applying iron that is available for turfgrass use will normally produce an immediate greening. The broad range of color of various turfgrass varieties may well be due to different chlorophyll contents. Iron materials are frequently applied to turfgrass to temporarily increase chlorophyll content and to green up the sward. The grass, other than becoming greener, apparently derives no growth benefits from iron (35).

For three years Kentucky bluegrasses (Poa pratensis L.) grown on the Colorado State University Campus at Fort Collins, Colorado,

had exhibited a dramatic difference in iron chlorosis expression.

Therefore, work was undertaken to investigate these color differences and the effect that soil iron has on chlorophyll (color) development in several Kentucky bluegrass varieties. Some workers (4, 6, 13, 16), have reported a positive correlation between iron and chlorophyll in different kinds of plants.

Generally, chlorotic leaves of plants have lower chlorophyll and iron content than normal green leaves, but as little work with chlorophyll and iron has been attempted with Kentucky bluegrass, especially as it related to varieties, this study was undertaken. The study reported here was an investigation of the iron-chlorophyll relationship of several Kentucky bluegrass varieties and blends.

REVIEW OF LITERATURE

Iron (Fe) is one of the most common elements in the surface of the earth. The total content in soils ranges from a low of 200 ppm to more than 10 per cent (26). Iron occurs in soils as oxides, hydroxides, and phosphates, as well as in the lattice structure of primary silicate and clay minerals (33). In soils small amounts of iron are released during the weathering of the primary and secondary minerals and a portion of this iron is absorbed by plants.

Brown and Tiffin (8) state that chelates are probably the natural means by which iron is absorbed by roots. However, they (42) had *also* suggested that absorption is a metabolic process, and ferric iron is reduced to the ferrous form by some carrier compound in the roots. Relatively large amounts of ferrous iron in the epidermis and vascular bundles of corn roots were found by Kilman (25). He suggested that roots changed the ferric form to the ferrous state before absorption and translocation.

Plants can absorb iron as either ferric (Fe^{+++}) or ferrous (Fe^{++}) ions. However, iron is physiologically utilized only in the ferrous state (2).

Within the plant iron functions in chlorophyll synthesis and as a constituent of certain enzymes in the respiratory system. Iron is not a constituent of chlorophyll, but it is required for chlorophyll synthesis. Thus, turfgrass color as related to chlorophyll is influenced

by the level of iron available to the plant. Chlorosis of the turf occurs when there is a deficiency of iron in the plant (11). Also, iron is a constituent of respiratory enzymes such as catalase, peroxidase, and cytochrome oxidase (2).

Iron is the micronutrient most commonly deficient in turf (31). An iron deficiency is usually the result of inability of plants to absorb enough to satisfy their needs rather than an absence of the element in the soil.

Total iron content of the soil is of no value in diagnosing iron deficiencies (26). And, it has been reported (15, 31) that the total soil iron does not reflect the iron supplying power of the soil (plant available iron). Lindsay and Norvell (30) have recently developed a test (DTPA soil test) that will measure the plant available iron in soil.

Deficiencies of iron are most common in soils that are (a) alkaline; (b) high in phosphate, manganese (Mn), zinc (Zn), or arsenic (As) (40); (c) high in organic matter content; (d) waterlogged; or (e) excessively thatched. Rediske and Biddulph (37) believe that three factors affect chlorosis by affecting the absorption of iron from the soil. These factors are pH, chemical form of the iron and phosphorus concentration.

Plant iron chlorosis is quite common in many crop and ornamental plants, especially turfgrass growing on alkaline and calcareous soils (43). In an experiment, Ganji (17) showed that chlorosis of apple trees

was due to an iron deficiency related to lime and an alkaline reaction of the soil.

Follett (15) reported that available iron usually increases as the soil pH decreases. Also, Rediske and Biddulph reported (37) that iron uptake increases as the pH decreases. Foret (16), while working with azaleas, found that soil acidity promoted absorption and utilization of iron, and a pH of 4 to 5 was optimum for these functions. Kock (26) and Altman (1) reported that under conditions of high soil pH or high lime content iron chlorosis of turf grasses can be observed. Throne et al (41) felt that high soil pH is one of the most important factors in producing plant chlorosis. The availability of iron for plant uptake according to Ludwick (31) is at a minimum in the pH range of 7.5 - 8.0.

However, results obtained by Olson (34) indicate that soil pH is not the only factor determining the solubility of iron in soils and in the ability of soils to supply iron to plants. He noted that at least one other factor was the amount of free iron oxide in the soil. In general according to various works (26, 34), iron deficiencies will most likely occur in soils high in pH and/or carbonates. It was observed by O'Conner (33) that iron chlorosis in field crops was induced by the bicarbonate ion in the irrigation water. Also, Kock (26) noted that bicarbonate tended to increase the severity of plant iron chlorosis. The incidence of iron chlorosis in soybeans was related more to the

phosphorus (P) and calcium (Ca) concentration in the solutions than to the HCO_3^- concentration (7), and the effect of HCO_3^- on the development of iron chlorosis appeared to be one which is indirect rather than direct. It has been suggested that (7, 26) carbon dioxide fixation by plant roots was responsible for iron chlorosis by inactivation iron within the plant.

Iron deficiencies are quite pronounced on some calcareous soils and a high level of soil phosphorus in some instances has been related to iron chlorosis (43). Phosphorus may interfere with utilization of iron; as the phosphorus concentration in soil is increased iron absorption is retarded (28, 37). According to Biddulph and Woodbridge (9) high phosphorus content of soils will decrease iron absorption. Olson (34) observed that chlorotic plants have a higher phosphorus content than normal green plants as well as a high P:Fe ratio. Others (4, 12, 13) have reported the same high P:Fe ratio of chlorotic leaves. Ludwick (31) suggested that iron deficiency may be induced or accentuated by heavy phosphorus fertilization. This induced deficiency is apparently due to a physiological antagonism within the plant itself which inactivates a portion of the absorbed iron (31). Pocklington (35) suggests that the P:Fe ratio of grass tissues may be a causal factor for the lower efficiency of iron for chlorophyll development resulting in lighter colored grasses.

In addition to iron, there are many other elements that contribute to chlorophyll synthesis and/or color development. Hewitt (20) noted

that sulfur (S), Ca, Copper (Cu), and Zn can all cause chlorosis. In another report (19) he suggested that molybdenum (Mo) consistently accentuated chlorosis in the presence of Mn, chromium (Cr), Zn, and Cu. He also noted the importance of nitrogen (N) and Mo in iron metabolism.

Plants growing in soils with high manganese (Mn) content have more severe chlorosis than those grown on soils with normal levels of this element. Somers and Shive (39) suggested that because of higher oxidation-reduction potential of Mn than Fe, manganese can oxidize iron to an inactive ferric state. O'Connor (33) also mentioned that the iron can be inactivated in plant tissues by an excess of Mn, which acts as an oxidizing agent (33).

It has been reported (39) that tissue of chlorotic plants have higher Mn:Fe ratios than normal green leaves. But, they (39) noted that Mn:Fe ratios alone are not a very important factor in the development of chlorosis symptoms, for, if the amount of iron and Mn decreases but the ratio stays constant, then the chlorosis symptoms will appear. Throne et al (41) believe that large amounts of available Mn are not an important factor for inducing iron chlorosis on most high lime soils nor in plants suffering from lime induced chlorosis. In an experiment (27) where dwarf kidney beans and tomatoes were grown in complete nutrient solutions containing various concentrations of iron and Mn, it was found that the Mn:Fe ratio in nutrient solution must be within a definite range in order to avoid deficiency symptoms in the plants.

The effect of ions such as Fe, Cu, and Zn on plant chlorosis has been discussed by different workers (4, 15, 27). It has been suggested that iron deficiencies (imbalance) observed on many Florida soils probably result from an accumulation of Cu in these soils after years of Cu application in sprays and fertilizers (43). Work done by Lindsay et al (29) and Smith and Specht (38) with iron has indicated that adding Zn to certain soils interferes with the iron metabolism in plants.

A low Ca:K ratio in chlorotic tissue has been reported by Dekock and Hall (12) and Iljin (21) while the ratio of Ca:K in normal leaves is higher. Potassium (K) has been reported to compete with iron in the structure of enzymes necessary to produce chlorophyll (3).

Also, positive correlations have been reported between iron content in plant tissue and chlorophyll (16, 26, 37, 43); however, Olson (34) has shown that only a particular fraction of iron is related to chlorophyll. Brown and Holmes (4) suggest that iron chlorosis is the failure of plant tissue to absorb iron or to utilize it. Iron chlorosis does not definitely establish a deficiency of iron. Sometimes there is not enough useable or active iron in a plant and it shows chlorosis while chemical tissue analysis shows a reasonable amount of iron in that plant. Furthermore, Jackson and Pertli (22) believe that luxury consumption of iron may have caused conflicting correlations.

Price and Carell (36) while working with Euglena gracilis reported that limited iron for chlorophyll synthesis may cause chlorosis, and

this is the reason for the presence of chlorosis in some plants, but not in others, even if both groups were planted in a similar nutrient buffer, containing two different levels of available iron.

The relationship between color and chlorophyll content in Seaside creeping bentgrass (Agrostis palustris Huds.) and Highland colonial bentgrass (Agrostis tenuis Sibth.) has been studied by Madison and Anderson (32). They suggested that the amount of chlorophyll in plant tissue has some influence on the grass color intensities. Turf response to applications of iron containing materials was measured by Deal and Engel (11). They noted that a temporary improvement in color was obtained by adding low amounts of iron to turf on low fertility soils, but in highly fertile soils, applications of iron to soil did not make a significant improvement in color of grasses.

In explaining plant iron chlorosis, Throne et al (41) mentioned that the oxidation of iron to the ferric state and possible inactivation in protein combinations followed by a disturbance in the protective protein-chlorophyll combination appears as a plausible concept to explain part of the relationship involved in chlorosis. A lack of oxygen in the root zone reduces plant chlorosis (26). This may be related to the lack of oxygen to convert ferrous (Fe^{++}) iron to the ferric (Fe^{+++}) state. Kock (26) reports that iron and manganese availability may be increased almost to the point of toxicity in soils with poor aeration.

Among other factors involved in Fe-chlorosis are cool temperature, and high soil moisture (31). Throne et al (41) do not believe that

these factors prevent iron uptake, but believe that these factors apparently inactivate iron within plants.

Iron absorption by soybeans has been studied by Brown et al (6, 8), and they reported that different varieties of soybean have different rates of root absorption of iron. Also, they reported that when chlorotic plants with a low iron absorption capacity were grown on root stock with a higher iron uptake the plants had greener leaves.

According to Butler (10) the basis of choosing Kentucky bluegrass varieties for most cool-humid regions, is the expected and desired qualities of this grass. He also mentions that a dark green color, which is especially important to the homeowner can be obtained by applications of nitrogen and iron.

Iron is the only micronutrient commonly considered necessary for the production of an acceptable turf. Iron fertilization is most common on intensively cultured turfs, such as bentgrass (Agrostis L.) and bluegrass (Poa L.). In addition to fertilization with iron containing materials, the application of an acidifying fertilizer may correct an iron deficiency caused by alkaline conditions (2).

Carriers utilized to correct an iron deficiency include (a) ferrous sulfate, (b) ferrous ammonium sulfate, (c) ferrous oxalate, and (d) chelated iron (43). These are water soluble iron carriers that correct the immediate iron deficiencies within the plant. There is no long term effect, however, because they are (a) quite water-soluble and subject to loss by leaching or (b) converted to insoluble and

unavailable forms, particularly under alkaline conditions (43). Foliar application of iron sulfate is frequently used to correct iron deficiencies in turf (31).

Iron can be combined with an organic compound to form a stable compound in the soil or synthetically to supply a source of iron for use on turf and other crops (chelated iron). The iron on the stable organic complex is readily exchanged with cations on the root surface and absorbed by the turfgrass plant. Chelated iron materials have a longer residual response in the soil than the water soluble compounds such as iron sulfate (43). Activated sewage sludge is also a source of naturally chelated iron and it can be used to correct iron chlorosis on turfgrass (43).

MATERIALS AND METHODS

Experimental plots of 25 varieties and 5 blends of Kentucky bluegrass (Poa pratensis L.) grown at the W. D. Holley Plant Environmental Research Center at Colorado State University, Fort Collins, Colorado, had for 3 summers shown dramatic varietal differences in the degree of chlorosis. Application of iron containing fertilizer in an adjoining area revealed that the chlorosis of Kentucky bluegrass could be corrected with either foliar or soil applications of iron.

Each variety and blend in this experiment was replicated 3 times in a randomized complete block design. The plots had been established for 3 years, and the varieties had all been established from seed except for Warren's A-20 which had been vegetatively propagated.

At the time of seeding in 1971, 1.61 lbs of N/1000 ft² (8 g of N/m²) as 16-20-0 fertilizer was applied as a starter fertilizer. During 1972, three applications (spring, summer, and fall) of 2 lbs of N/1000 ft² (10 g of N/m²), and in 1973, 1 lb of N/1000 ft² (5 g of N/m²) of NH₄NO₃ (34-0-0) were applied (three applications as above). In 1974, two applications of 1 lb of N/1000 ft² (5 g of N/m²) of 34-0-0 was applied in May and July.

The plot size was 10 ft x 10 ft (3^m x 3^m), half of each was mowed to 1 1/2 inches (3.81 cm) and the other half at 3/4 inch (1.90 cm).

The tissue used in this work was all taken from the area cut at 3/4 inch (1.9 cm).

Plant samples were collected for chlorophyll and iron determinations on September 25, two days after the previous mowing on September 23. A push type mower set at 3/4 inches was used to collect the clippings. Immediately after sampling the plant tissue, samples were rinsed with distilled water, then were oven dried at 150°F (65.5°C) for 24 hours, and were then stored in plastic bags to prevent moisture absorption. The plant samples were then ground using a stainless steel grinder and a 40 mesh sieve.

Chlorophyll was extracted using 250 ml of methanol and .25 g of plant material. Extraction was done at room temperature and the amount of chlorophyll present was determined according to Johnson (24), using a Spectronic 20 to determine per cent of transmittance. This procedure for measuring turfgrass color is simple and economical; and also color assessed by this procedure is an intrinsic measure of color, made quantitatively, objectively and independently of yield, thatch, texture, or density of the turf. The wave length used for this determination was 660 nm.

In order to determine the total iron concentration in the plant samples, one gram of the sample was placed in a 100 ml volumetric flask with 10 ml of HNO_3 and kept over night. The samples were then digested using a mixture of HNO_3 - H_2SO_4 - HClO_4 in a proportion of

10:1:14 respectively [procedure outlined by Jackson (23)]. The digestion was carried out at 180°C - 200°C , then the material was filtered and the volume was brought to 100 ml using dionized distilled water. The iron concentration was then determined by using a Perkin-Elmer Model 303 Atomic Absorption Spectrophotometer.

At the time that the clippings were collected, core samples of soil to a 2 inch (5 cm) depth were also collected, using a stainless steel core sampler. Five samples from each plot were composited. The samples were air dried at room temperature (22°C) for chemical analysis. The air dried samples were ground using a grinder with porcelain pestle and mortar and a 2 mm sieve.

The soil pH was determined on the saturated paste. An Ionalyser, Model 80, Digital pH Meter, was used to determine pH. The electrodes were allowed to equilibrate with paste for 20 minutes.

To measure the soil available iron, a micronutrient soil test was made using 0.005 M-D. T. P. A. (diethylene triaminepenta-acetic acid), 0.01 M- CaCl_2 , and 0.1 M-T. E. A. (triethanolamine) as a buffer at pH 7.30 (31). Using milk bottles and a shaker, the mixture was then oscillated at room temperature for two hours with 120 oscillations per minute. The suspension was filtered, and the iron determined directly from the filtrate by atomic absorption spectrophotometry. This is a very fast and easy method and according to Lindsay (30), it can be used equally well on calcareous and noncalcareous soil.

(Details related to data are shown in appendix section.)

RESULTS AND DISCUSSION

Different varieties and blends of Kentucky bluegrass seem to respond differently to soil iron even if the available iron in the soil is the same. To determine why this variability exists, research was conducted to ascertain the difference between 25 varieties and five blends of Kentucky bluegrass. Plant variability as to iron and chlorophyll contents was determined; also, soil available iron and pH were established.

The result of these determinations is summarized in Table 1. Each value in Table 1 is the mean of 3 replicates of each variety or blend. Color ratings are observed visual determinations (with 10 a value of being equal to the darkest green).

It has been commonly thought that plant available iron was closely related to soil pH. As it was pointed out earlier (1, 16, 26, 31, 34, 37), with an increase in soil pH, Fe availability decreases. As the reaction rises above a pH of 7.0, the situation becomes more complex.

In this work the soil pH and soil available iron of the individual plots were not significantly different, and when pH values are plotted (Fig. 1) against soil available iron, a significant correlation did not occur (0.004 correlation coefficient "r" between these two variables, Table 3). As would be expected these results indicate only slight

Table 1 - Soil and plants analysis data, a measure of soil pH and available iron, plant total iron, chlorophyll content, iron uptake efficiency, and color ratings.

(a) Variety	pH	ppm Soil-available iron	ppm Plant-total iron	mg/g Chlorophyll	(b) Iron-uptake efficiency	(c) Color rate
Pennstar	7.46	13.41	203	2.54	15.6	8
Fylking	7.50	17.93	270	2.88	15.0	9
Adelphi	7.51	13.87	271	2.27	19.5	10
Prato	7.40	13.82	250	2.75	18.7	9
S.21	7.48	12.66	202	2.27	16.1	7
Windsor	7.53	13.76	268	2.93	19.9	10
Common #1	7.55	15.26	236	2.60	16.0	8
Common #2	7.47	12.66	226	2.55	17.8	7
Nugget	7.47	11.83	165	1.53	14.2	2
Primo	7.42	9.78	183	2.42	19.8	7
Gearry	7.54	13.61	246	2.69	18.7	8
Delta	7.53	13.50	248	2.73	20.0	8
Newport	7.58	13.06	224	2.62	18.4	9
Park	7.54	14.10	198	2.08	14.0	4
Melle	7.42	13.60	211	2.45	15.4	7

Table 1 - (cont'd)

(a) Variety	pH	ppm Soil-available iron	ppm Plant-total iron	mg/g Chlorophyll	(b) Iron-uptake efficiency	(c) Color rate
Ill. 38-17	7.50	13.83	296	3.56	21.9	10
Sodco	7.52	15.82	273	3.20	19.2	10
Kenblue	7.53	13.33	262	2.69	20.0	8
Arboretum	7.48	14.20	164	2.07	12.2	3
Merion	7.50	15.86	177	2.33	12.0	6
Code 95	7.53	13.56	224	2.63	17.2	8
Sydsport	7.42	10.53	266	2.90	25.3	10
Baron	7.50	12.96	233	2.53	19.8	8
Warren's A-20	7.56	9.96	172	2.16	17.8	5
Warren's A-34	7.58	12.30	155	1.68	12.5	2
Fylking + Pennstar Nugget	7.41	16.86	175	2.17	10.5	5
Park + Delta + Newport	7.45	13.63	171	1.74	12.9	3

Table 1 - (cont'd)

(a) Variety	pH	ppm Soil-available iron	ppm Plant total iron	mg/g Chlorophyll	(b) Iron-uptake efficiency	(c) Color rate
Windsor + Merion	7.49	16.43	262	2.72	16.7	10
Merion + Delta	7.51	9.12	176	2.31	20.4	7
Common + Kenblue	7.47	13.68	275	3.65	21.1	10
mean	7.49	13.18	222	2.47	17.2	7.3
Difference	insigni- ficant	insigni- ficant	**	**	*	--

(a) = Each value is a mean of three replicates.

(b) = Ratio of plant iron to soil available iron content.

(c) = Color values are visual estimates: 2 = lightest, 10 = darkest.
Made on September 1974.

Table 2 - Soil analysis of a composite sample taken at the time of clipping harvest.

Texture	pH	Salts mmhos/cm	O.M. %	N ppm	P ppm	K ppm	Zn ppm	Iron ppm	Lime %
sandy clay loam	7.3	.6	2.8	9	13	303	.9	13.49	High

Table 3 - Correlation between 5 factors evaluated in studying the effect of iron on chlorophyll development.

r	pH	Soil available Fe	Plant total Fe	Chlorophyll	Fe uptake efficiency
pH	--	0.004	0.058	-0.009	0.013
soil available Fe	--	--	0.327	0.233	* -0.438
plant total Fe	--	--	--	** 0.903	** 0.678
chlorophyll	--	--	--	--	** 0.666
Fe uptake efficiency	--	--	--	--	--

* significant at .05

** significant at .01

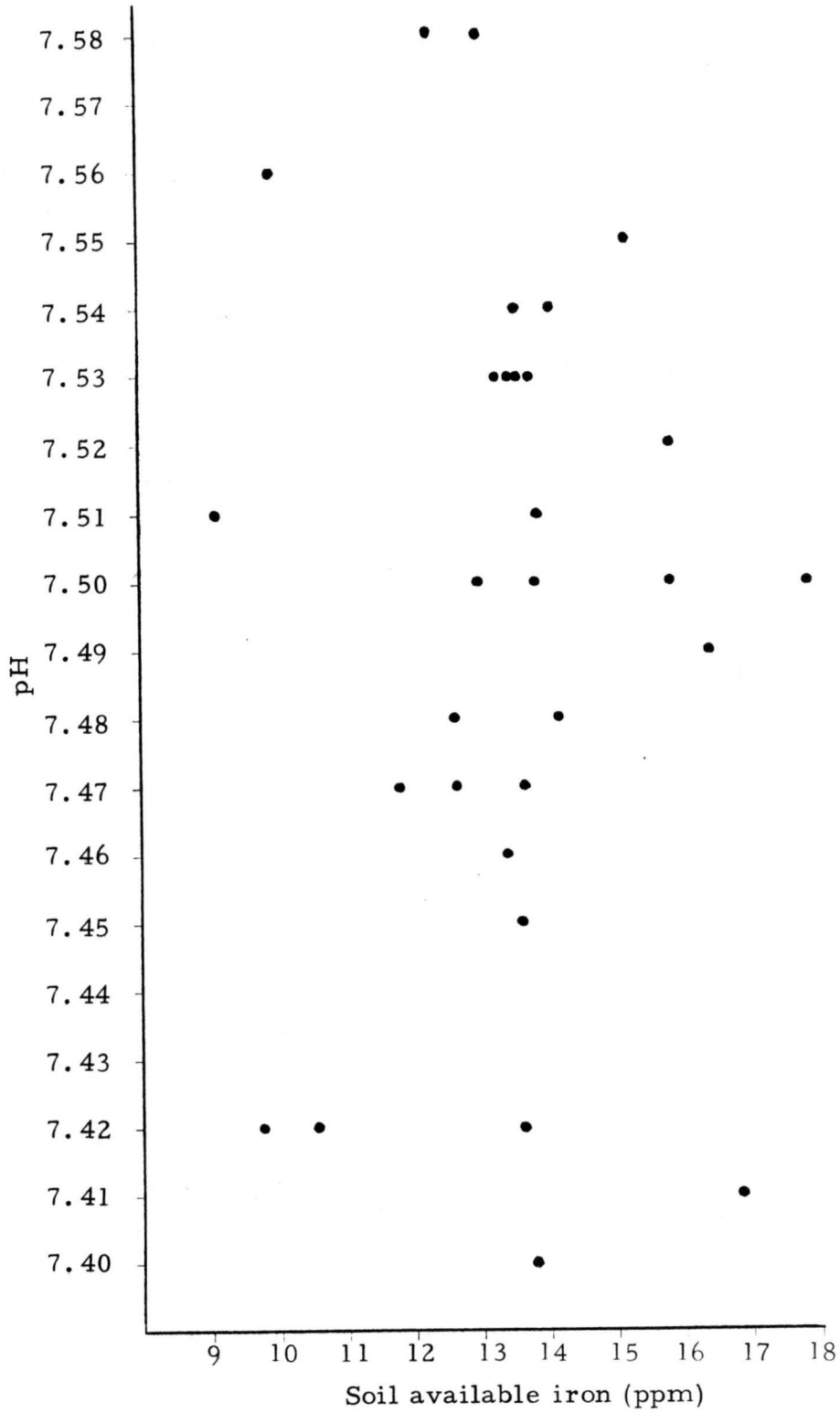


Fig. 1. Relationship of soil available iron to soil pH

differences between pH values (Table A-1), and soil iron available in samples collected from the plots. It was felt that the relatively high pH (7.9 mean of all samples) of this soil is at least one reason for the relatively low iron availability in the soil (Table 1). Also, as indicated in Table 3, a significant correlation between pH and other variables does not exist. A slightly higher relationship of pH to iron uptake and to plant total iron seems to be evident, but when statistically treated, this relationship was not significant. Even if soil pH does not have a significant correlation with soil available iron, it may exert a negative effect on iron absorption or on the iron stored in the plant.

Soil available iron levels, as pointed out earlier, were not significantly different between plots (Table 1); however, these levels were enough to satisfy the iron needs of certain varieties of grasses and produce turf that did not display chlorosis. The varietal differences and small correlation between plant iron and soil iron indicates that roots of different varieties are not the same in their ability of absorbing iron from soil.

Statistical analysis showed a significant difference ($P = .01$) between Kentucky bluegrass varieties and blends in their total content of iron. Fig. 3 shows the difference in total iron content between varieties. The significant difference in total iron content was determined using Honestly Significant Difference (H. S. D.) values.

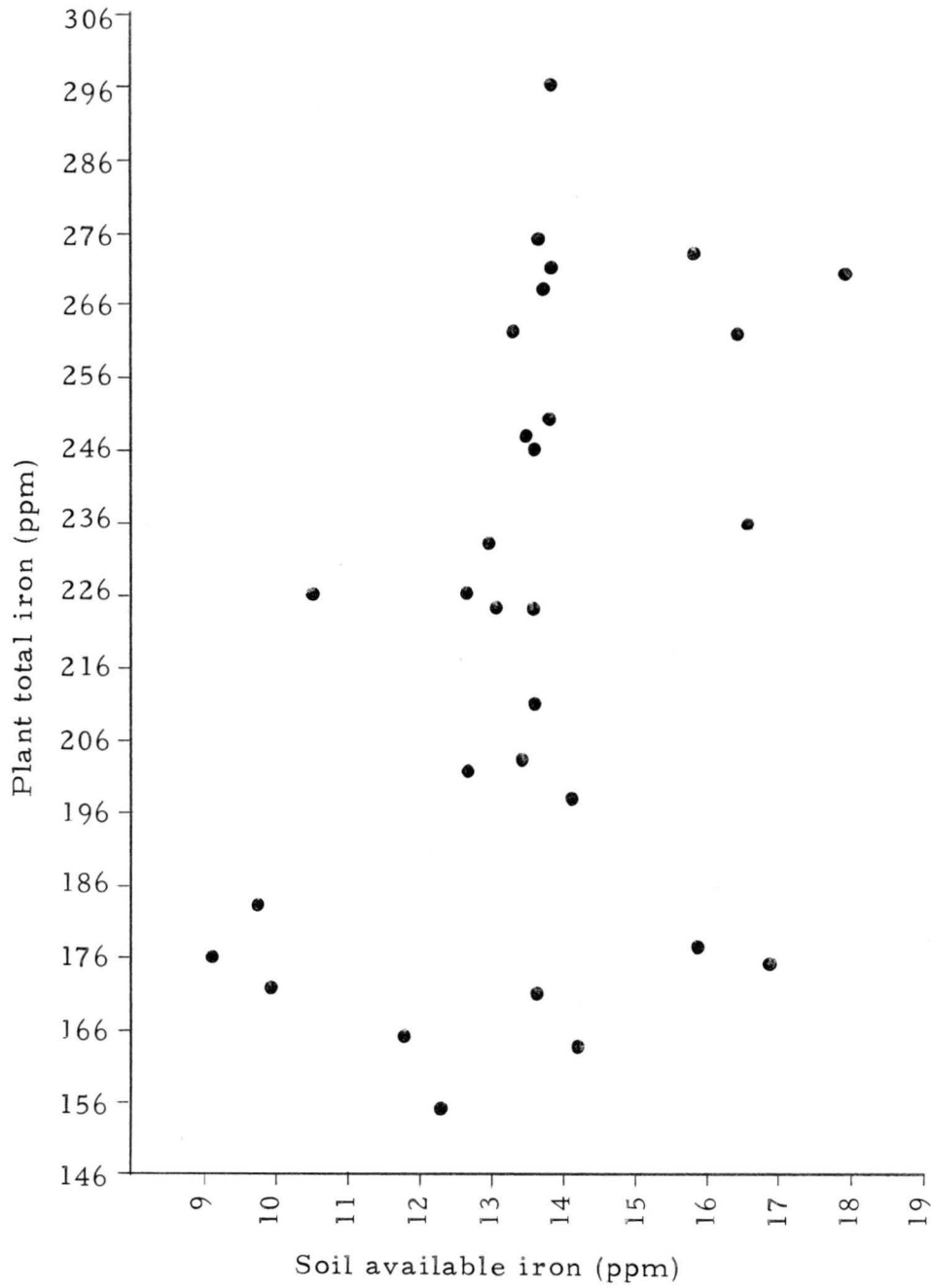
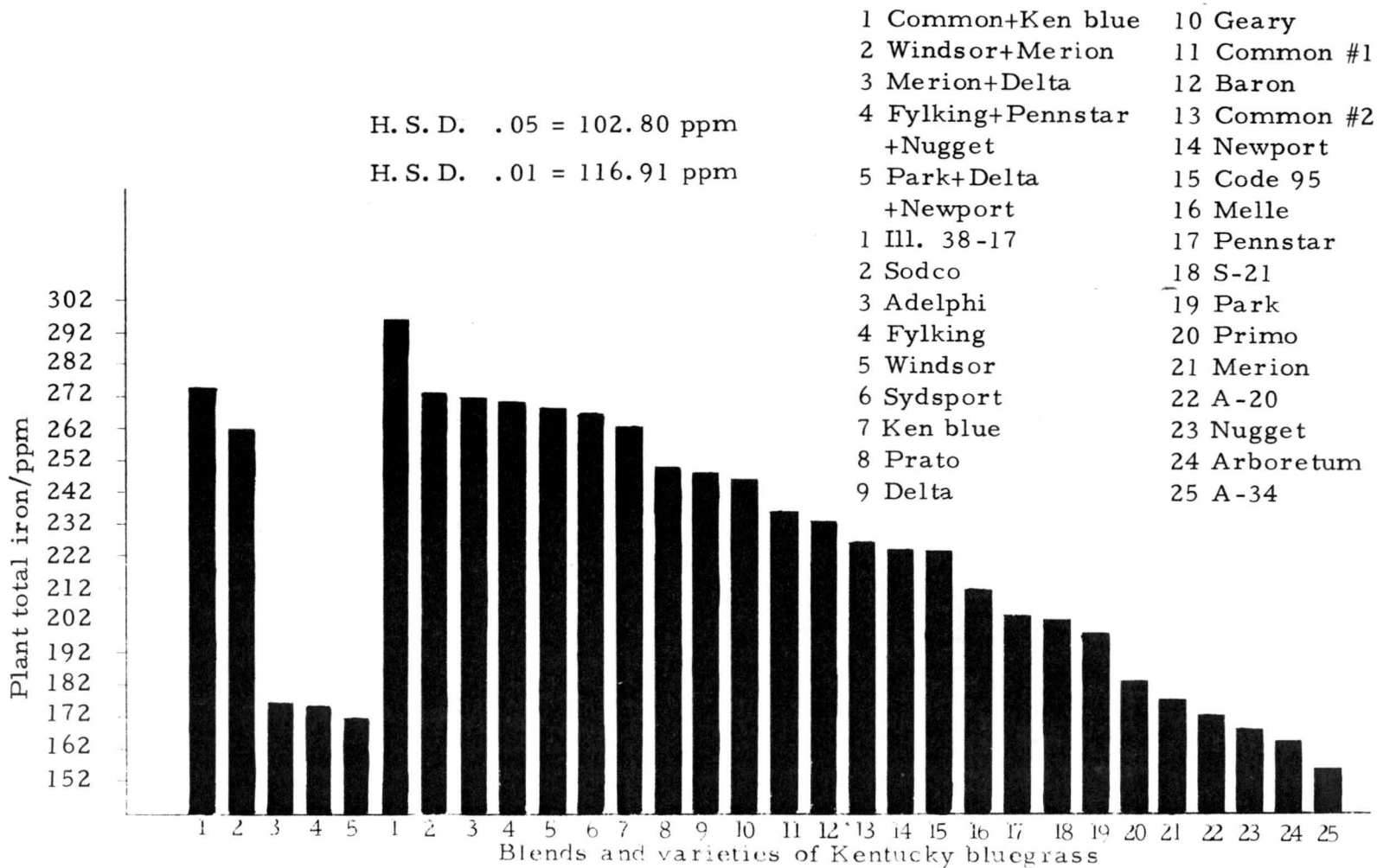


Fig. 2. Relationship of soil available iron to plant total iron.

Fig. 3. Varietal differences of plant iron content of 25 varieties
and 5 blends of Kentucky bluegrass.



A significant positive correlation existed between plant total iron and iron uptake efficiency. The iron uptake efficiency was calculated by dividing plant iron concentration by soil available iron concentration. This may show that some of these grasses are much more efficient in absorbing iron from the soil than others to meet their needs to produce acceptable color and little if any iron chlorosis.

Plant total iron showed a highly significant correlation with plant chlorophyll content ($r = **0.903$). This correlation is illustrated in Fig. 4. Some of these grasses seemed to have a much greater ability to utilize soil iron for chlorophyll formation.

In Fig. 5, the color values have been plotted against the chlorophyll content of the turfgrass. A positive correlation between visual color values and chlorophyll content is evident as indicated by the linear character between these two variables. It appeared that chlorophyll contents higher than 3.0 mg/g of clipping could be associated with darker green turfgrass (Fig. 5). Also Fig. 5 suggests that chlorophyll contents lower than 2.3 mg/g could be associated with a lighter green color in Kentucky bluegrass varieties. Correlation between color and chlorophyll content indicate that grasses with a high chlorophyll content should have a dark green color. This would tend to substantiate reports by Madison and Anderson (32) and Deal and Engel (11). Chlorophyll content appears to be at least one factor that influences the color difference between Kentucky bluegrass varieties and blends (Fig. 6).

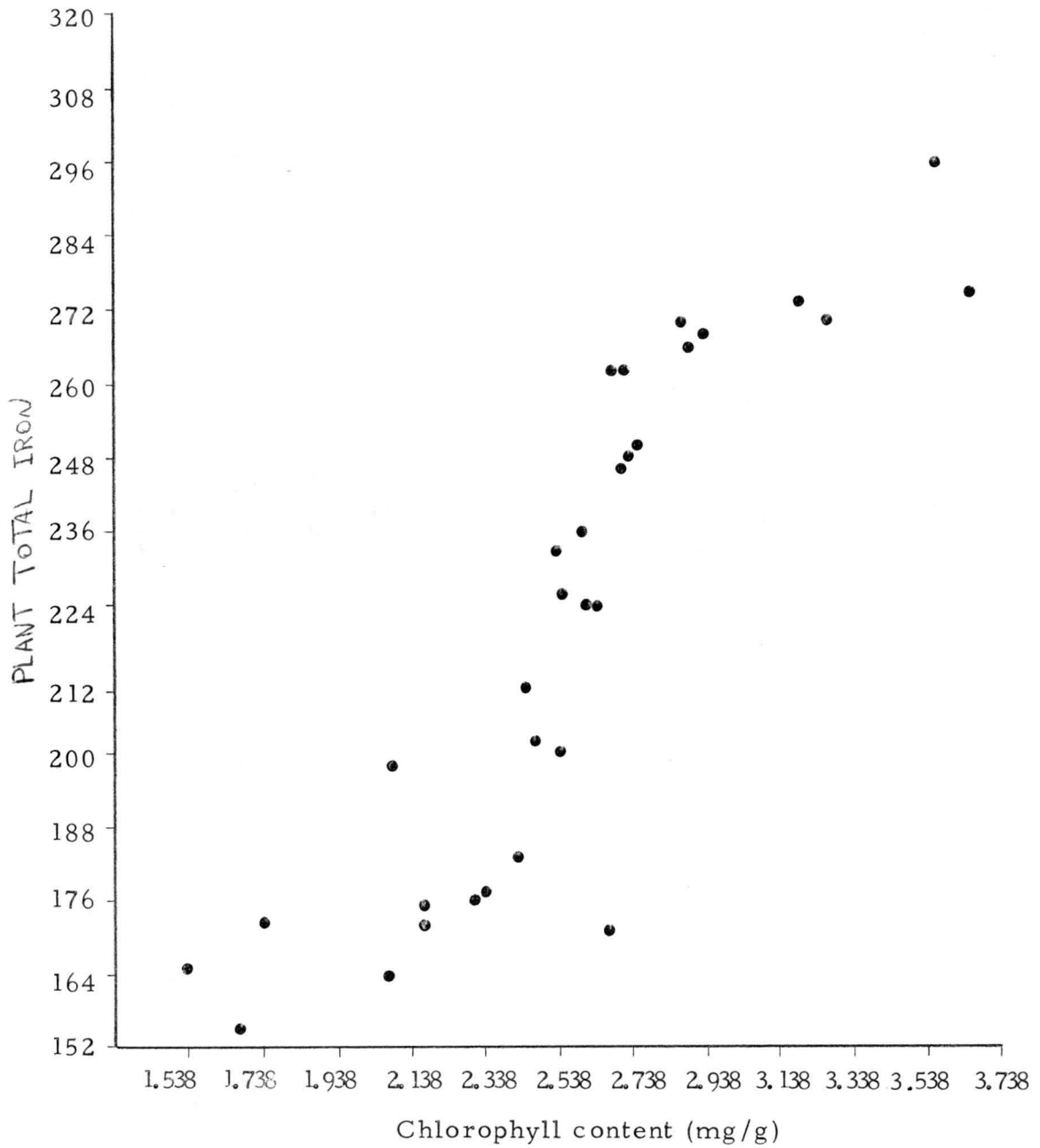


Fig. 4. Relationship of leaf chlorophyll content to plant total Fe.

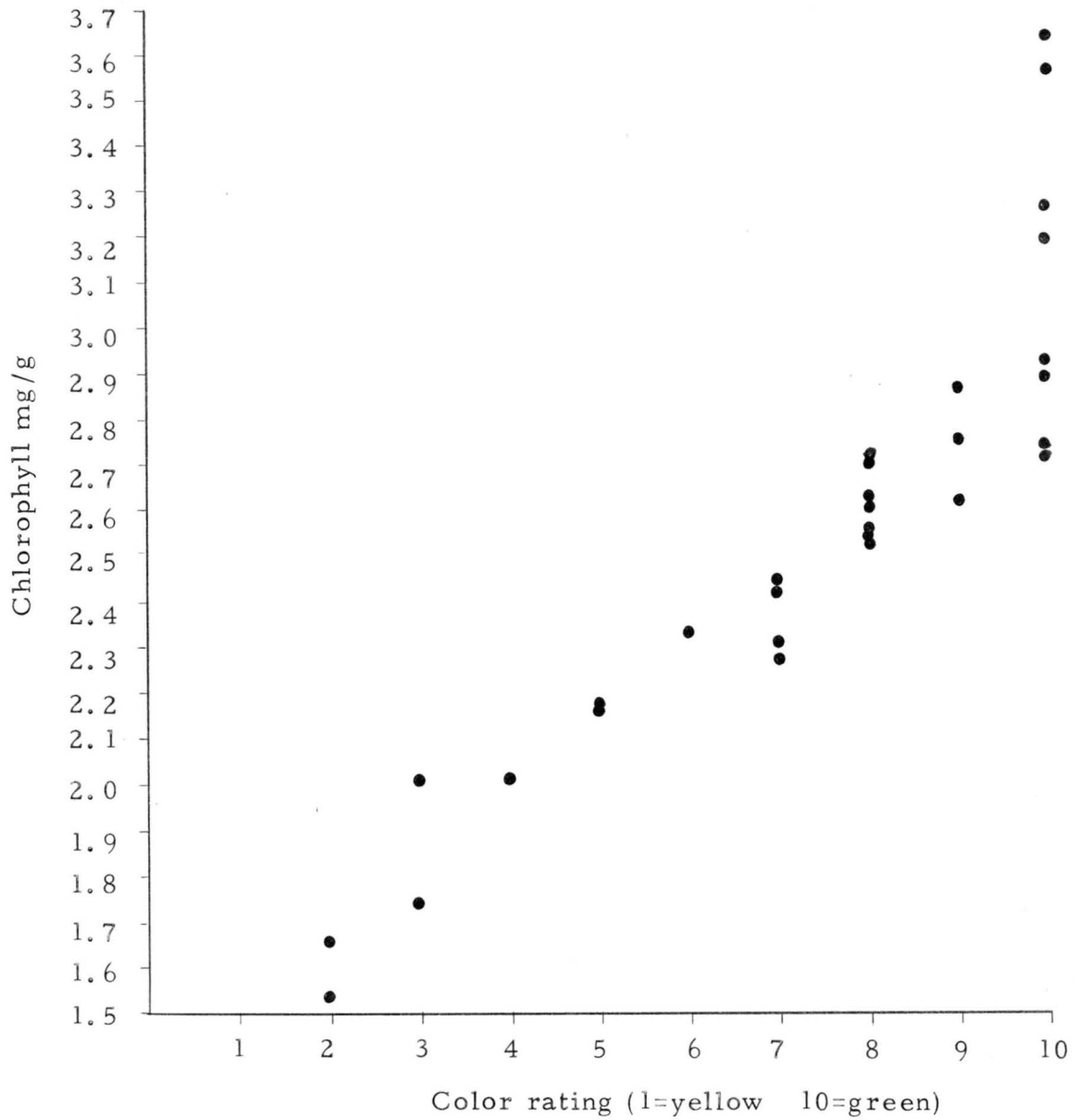
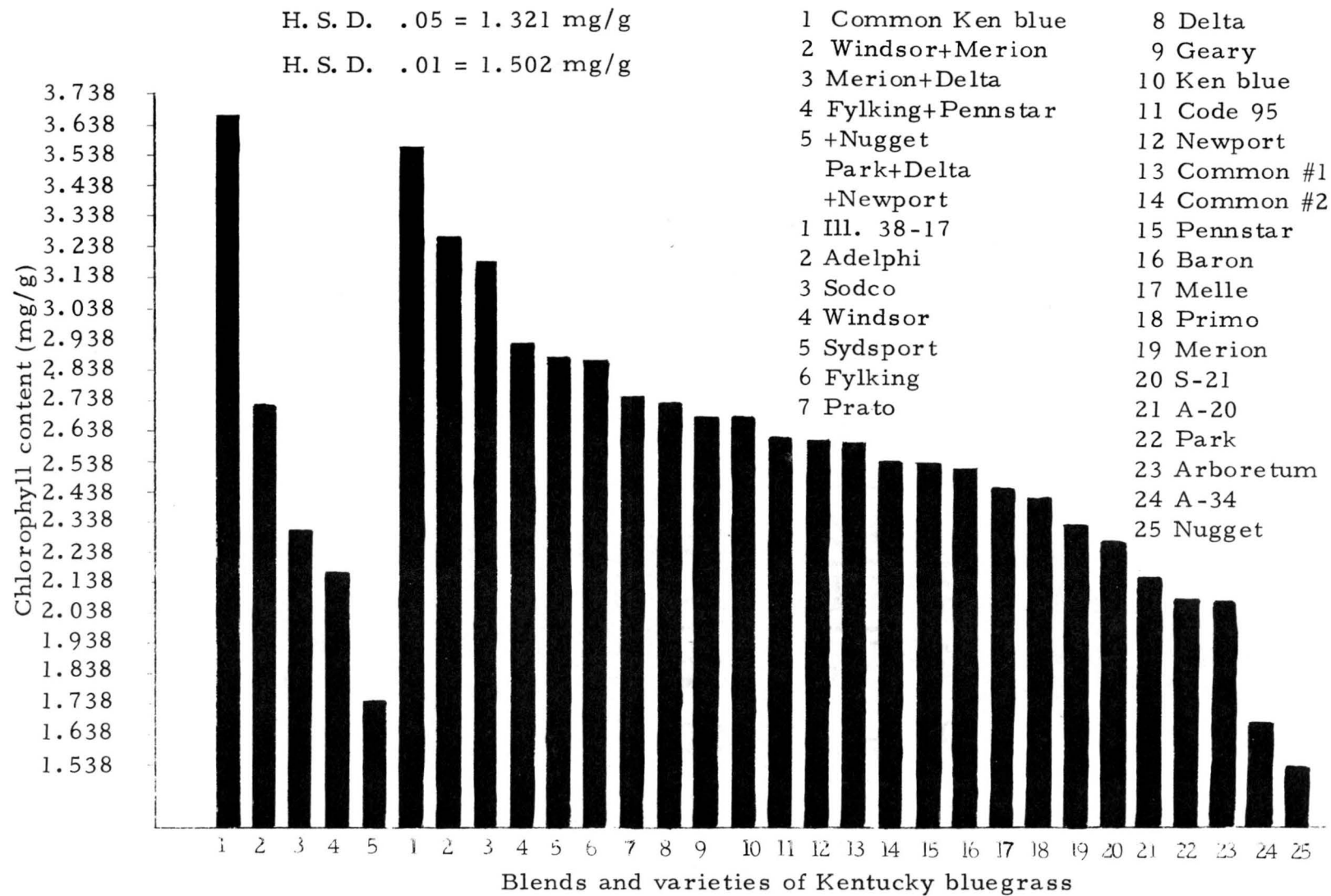


Fig. 5. Relationship of leaf chlorophyll content to color rating.

Fig. 6. Varietal differences of leaf chlorophyll contents
of 25 varieties and 5 blends of Kentucky bluegrass.





Statistical analysis showed that there was a significant difference in chlorophyll content between varieties and blends ($P = .01$).

Varietal differences in chlorophyll content determined by H. S. D. in Fig. 6 indicated that most of the varieties and blends used in this experiment are significantly different from at least one other variety or blend in their chlorophyll content value.

Certain varieties in this study remained green and aesthetically acceptable while others did not. Table 4 indicates that the varieties Adelphi, Windsor, Ill. Expt 38-17, Sodco, Sydsport, and the blends Windsor + Merion, and Common + Kenblue had an especially high iron uptake efficiency compared to the other varieties.

The varieties Warren's A-20 and A-34, Park, Arboretum, and the blends Fylking + Pennstar + Nugget, and Park + Delta + Newport showed a severe chlorosis. These varieties and blends had low iron uptake efficiency indexes (Table 5).

Table 4 - Comparison of 7 varieties of Kentucky bluegrass of acceptable green color.

Variety	ppm Soil-available Iron	ppm Plant-total Iron	mg/g Chlorophyll	Fe uptake efficiency	Rating
Adelphi	13.87	271	2.27	19.5	10
Windsor	13.76	268	2.93	19.9	10
Ill. 38-17	13.83	296	3.56	21.9	10
Sodco	15.82	273	3.20	19.2	10
Sydsport	10.53	266	2.90	25.3	10
Windsor + Merion	16.43	262	2.72	16.7	10
Common + Kenblue	13.68	275	3.65	21.1	10
average of data	13.18	222	2.47	23.6	7.3

Table 5 - A comparison of 7 varieties of Kentucky bluegrass with severe chlorosis.

Variety	ppm Soil-available iron	ppm Plant-total iron	mg/g Chlorophyll	Iron uptake efficiency	Color rating
Warren's A-20	9.96	172	2.16	17.8-	5
Fylking + Pennster + Nugget	16.86	175	2.17	10.5	5
Park	14.10	198	2.08	14.0	4
Park + Delta + Newport	13.63	171	1.74	12.9	3
Arboretum	14.20	164	2.07	12.2	3
Nugget	11.83	165	1.53	14.2	2
Warren's A-34	12.30	155	1.68	12.5	2
Average of all data	13.18	222	2.47	23.6	7.3

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APPENDIX

Table A-1 - Statistical Analysis of soil pH values.

VARIETIES	PENN- STAR	FYL- KING	ADEL- PHI	PRATO	S-21	WIND- SOR	COMMON #1	COMMON #2	NUG- GET	PRIMO	GEARY	DELTA	NEW- PORT	PARK	MELLE
R ₁	7.60	7.61	7.46	7.41	7.53	7.54	7.52	7.42	7.34	7.44	7.48	7.50	7.62	7.52	7.33
pH R ₂	7.22	7.53	7.51	7.30	7.33	7.41	7.53	7.51	7.48	7.44	7.54	7.48	7.52	7.50	7.38
R ₃	7.57	7.37	7.57	7.50	7.58	7.64	7.59	7.48	7.59	7.37	7.59	7.69	7.59	7.61	7.55
mean Σx/n	7.46	7.50	7.51	7.40	7.48	7.53	7.55	7.47	7.47	7.42	7.54	7.53	7.58	7.54	7.42
Source of Variation	d. f.		S.S.		M.S.		observed F		95%		required F		99%		
Total	89		.72		.01		--		--						
Block	2		.16		.08		--		--						1 < 1.65 < 2.03
Treat (varieties)	29		.21		.01		1		1.65		2.03				No significant differences existed
Error	58		.35		.01		--		--		--				

Table A - 1 - (cont'd)

VARIETIES	ILL. 38-17	SODCO	KEN- BLUE	ARBO- RETUM	MERION	CODE 95	SYD SPORT	BARON	A-20 WARREN'S	A-34 WARREN'S	FYL+ PENN+ NUG	PARK+ DEL+ NEWP	WIND+ MERION	MERION+ DELTA	COMMON KEN BLUE
R ₁	7.47	7.50	7.49	7.58	7.45	7.45	7.50	7.50	7.57	7.66	7.43	7.45	7.46	7.46	7.39
pH R ₂	7.46	7.50	7.55	7.36	7.47	7.53	7.27	7.44	7.52	7.49	7.26	7.46	7.37	7.53	7.49
R ₃	7.57	7.55	7.56	7.49	7.58	7.60	7.49	7.57	7.58	7.60	7.53	7.43	7.64	7.54	7.54
mean $\Sigma x/n$	7.50	7.52	7.53	7.48	7.50	7.53	7.42	7.50	7.56	7.58	7.41	7.45	7.49	7.51	7.47

Table A - 2 - Statistical analysis of soil available iron values.

VARIETIES		PENN- STAR	FYL- KING	ADEL- PHI	PRATO	S-21	WIND- SOR	COMMON #1	COMMON #2	NUG- GET	PRIMO	GEARY	DELTA	NEW- PORT	PARK	MELLE
Soil	R ₁	11.5	17.2	12.5	10.2	15.8	12.5	18.7	11.3	14.7	7.34	14.7	19.1	17.2	13.6	16.6
Avail- able Fe	R ₂	16.5	19.3	14.8	16.5	12.2	16.5	16.2	11.3	10.8	12.4	15.8	9.9	12.4	15.5	14.3
	R ₃	12.2	17.3	14.3	14.7	10.0	12.2	10.9	15.4	10.0	9.6	10.3	11.5	9.6	13.2	10.3
mean Σx/n		13.4	17.9	13.8	13.8	12.6	13.7	15.7	12.6	11.8	9.7	13.6	13.5	13.0	14.1	13.6
Source of Variation	d. f.		S.S.		M.S.		observed F		95%		required F 99%					
Total	89		1056		11.8		--		--		--					1.31 < 1.65 < 2.03
Block	2		181		90.6		--		--		--					no significant difference existed
Treat (varieties)	29		346		11.9		1.31		1.65		2.03					
Error	58		528		9.1		--		--		--					

Table A - 2 - (cont'd)

VARIETIES	38-17	SODCO	KEN BLUE	ARBO- RETUM	MERION	CODE 95	SYD SPORT	BARON	A-20 WARREN'S	A-34 WARREN'S	FYL- PENN+ NUG	PARK+ DEL+ NEWP	WIND+ MERION	MERION+ DELTA	COMMON +KEN BLUE
Soil R ₁	11.5	21.6	16.8	19.3	19.3	9.9	9.9	18.5	9.9	11.8	22.7	12.4	19.1	7.2	11.9
Avail- able Fe R ₂	17.2	16.9	12.4	11.8	16.8	17.2	10.9	9.2	12.4	12.9	17.4	17.2	19.3	11.8	17.9
R ₃	12.8	8.9	10.8	11.5	11.5	13.6	10.8	11.2	7.6	12.2	10.5	11.3	10.9	8.3	11.2
mean Σx/n	13.8	15.8	13.3	14.2	15.8	13.5	10.5	12.9	9.9	12.3	16.8	13.63	16.4	9.1	13.6

Table A - 3 - Statistical analysis of plant total iron values.

VARIETIES		PENN-STAR	FYL-KING	ADEL-PHI	PRA TO	S-21	WIND-SOR	COMMON #1	COMMON #2	NUG-GET	PRIMO	GEARY	DELTA	NEW-PORT	PARK	MELLE
Plant	R ₁	185	259	246	245	218	309	177	194	173	207	219	239	196	212	241
Total	R ₂	184	268	287	280	237	245	327	221	169	177	264	269	242	184	231
Iron	R ₃	242	285	282	226	153	252	204	263	155	165	256	236	235	198	162
mean																
$\Sigma x/n$		203	270	271	250	202	268	236	226	165	183	246	248	224	198	211

Source of Variation	d. f.	S. S.	M. S.	observed F	95%	99%	required F
Total	89	212251	2384	--	--	--	5.1 > 2.03 > 1.65
Block	2	838	419	--	--	--	So there was a significant difference between varieties in their Fe-content.
Treat (varieties)	29	152020	5242	5.1	1.65	2.03	H. S. D. .05 = $\sqrt{\frac{1024}{3} \times 5.566} = 102.80$
Error	58	59392	1024	--	--	--	H. S. D. .01 = $\sqrt{\frac{1024}{3} \times 6.33} = 116.91$

Table A - 3 - (cont'd)

VARIETIES	ILL. 38-17	SODCO	KEN. BLUE	ARBO RETUM	MERION	CODE 95	SYD SPORT	BARON	A-20 WARREN'S	A-34 WARREN'S	FYL+ PENN+ NUG	PARK+ DEL+ NEWP	WIND+ MERION	MERION+ DELTA	COMMON +KEN BLUE	
Plant	R ₁	276	266	291	159	165	211	289	254	181	125	208	163	311	201	290
Total	R ₂	294	308	257	158	153	270	270	186	173	143	205	175	240	166	260
Iron	R ₃	318	245	238	177	213	241	240	259	162	197	114	177	236	163	277
Mean $\Sigma x/n$		296	273	262	164	177	224	266	233	172	155	175	171	262	176	275

Table A - 4 - Statistical Analysis of plant chlorophyll content values.

VARIETIES	PENN-STAR	FYL-KING	ADEL-PHI	PRATO	S-21	WIND-SOR	COMMON #1	COMMON #2	NUG-GET	PRIMO	GEARY	DELTA	NEW-PORT	PARK	MELLE	
Chloro- phyll mg/g	R ₁	1.9	3.0	3.0	2.2	1.9	2.2	2.8	2.1	1.1	2.7	2.7	2.9	2.7	1.8	2.4
	R ₂	2.4	2.5	3.3	2.9	2.6	2.7	2.4	2.8	1.7	2.8	2.7	2.3	2.3	2.4	2.4
	R ₃	3.1	2.8	3.4	3.1	2.2	3.8	2.6	2.7	1.7	1.7	2.6	2.9	2.7	1.9	2.4
mean Σx/n		2.5	2.8	3.2	2.7	2.2	2.9	2.6	2.5	1.5	2.4	2.6	2.7	2.6	2.0	2.4

Source of Variation	d. F.	S.S.	M.S.	observed F	95%	required F	99%
Total	89	32.104	.361	--	--	--	4.3 > 2.03 > 1.65
Block	2	1.132	.566	--	--	--	--
Treat (varieties)	29	21.152	.729	4.3	1.65	2.03	H.S.D. .05 = $\sqrt{\frac{.169}{3}} \times Q_{.05, 30, 58} = 1.32 \text{ mg/g}$ H.S.D. .01 = $\sqrt{\frac{.169}{3}} \times Q_{.01, 30, 58} = 1.50 \text{ mg/g}$
Error	58	9.820	.169	--	--	--	--

Table A - 4 - (cont'd)

VARIETIES	ILL. 38-17	SODCO	KEN BLUE	ARBO- RETUM	MERION	CODE 95	SYD- SPORT	BARON	A-20 WARREN'S	A-34 WARREN'S	FYL+ PENN+ NUG	PARK+ DEL+ NEWP	WIND+ MERION	MERION +DELTA	COMMON +KEN BLUE	
Chloro- phyll mg/g	R ₁	3.5	3.5	2.9	2.4	2.7	2.9	3.5	2.6	2.6	2.1	2.4	2.0	3.1	2.7	4.1
	R ₂	3.0	2.9	2.0	1.8	2.1	2.5	3.0	2.5	1.8	1.0	1.6	1.2	2.1	1.7	3.2
	R ₃	4.1	3.1	3.1	1.9	2.1	2.4	2.1	2.3	2.0	1.8	2.4	1.9	2.8	2.4	3.6
mean $\Sigma x/n$		3.5	3.2	2.6	2.0	2.3	2.6	2.9	2.5	2.1	1.6	2.1	1.7	2.7	2.3	3.6

Table A - 5 - Statistical analysis of plant iron uptake efficiency.

VARIETIES	PENN-STAR	FYL-KING	ADEL-PHI	PRATO	S-21	WIND-SOR	COMMON #1	COMMON #2	NUG-GET	PRIMO	GEARY	DELTA	NEW-PORT	PARK	MELLE
Iron R ₁	16.0	15.0	19.6	24.0	13.7	24.6	9.4	17.1	11.7	28.2	14.8	12.5	11.3	15.5	14.5
Uptake R ₂	11.1	13.8	19.3	16.9	19.4	14.7	20.1	19.5	15.6	14.2	16.7	27.1	19.5	11.8	16.1
Efficiency R ₃	19.8	16.4	19.7	15.3	15.3	20.6	18.7	17.0	15.5	17.1	24.7	20.5	24.4	15	15.7
mean Σx/n	15.6	15.0	19.5	18.7	16.1	19.9	16.0	17.8	14.2	19.8	18.7	20.0	18.4	14.1	15.4
Source of Variation	d. f.		S.S.		M.S.		observed F		95%		required F		99%		
Total	89		2215.301		24.891		--		--		--		--		2.03 > 2 > 1.65
Block	2		168.548		84.274		--		--		--		--		There is significant difference with 95% probability.
Treat (varieties)	29		1003.987		34.620		2*		1.65		2.03		--		
Error	58		1042.766		17.979		--		--		--		--		

Table A - 5 - (Cont'd)

VARIETIES		ILL. 38-17	SODCO	KEN BLUE	ARBO- RETUM	MERION	CODE 95	SYD- SPORT	BARON	A-20 WARREN'S	A-34 WARREN'S	FYL+ PENN+ NUG	PARK+ DEL+ NEWP	WIND+ MERION	MERION+ DELTA	COMMON +KEN BLUE
Iron	R ₁	24	12.3	17.3	8.2	8.5	21.3	29.1	16.3	18.2	10.5	9.1	13.1	16.2	27.9	24.2
Uptake	R ₂	17.0	18.2	20.7	13.3	9.1	12.7	24.7	20.2	13.9	11.0	11.7	10.1	12.4	14.0	14.5
Efficiency	R ₃	24.8	27.3	22.0	15.3	18.5	17.7	22.2	23.1	21.3	16.1	10.8	15.6	21.6	19.4	24.7
mean $\Sigma x/n$		21.9	19.2	20	12.2	12.0	17.2	25.3	19.8	17.8	12.5	10.5	12.9	16.7	20.4	21.1

										10'
	1	2	3	4	5	6	7	8	9	10
R ₁	11	12	13	14	15	16	17	18	19	20
	21	22	23	24	25	26	27	28	29	30
	8	23	19	28	29	13	17	3	30	4
R ₂	20	12	9	1	6	25	26	14	22	11
	7	15	21	10	16	27	24	2	5	18
	24	6	12	1	18	22	9	13	16	7
R ₃	15	28	5	27	11	23	20	3	21	25
	26	4	2	17	8	30	19	29	10	14

Fig. A-1. Experimental plots of 25 varieties and 5 blends of Kentucky bluegrass.

<u>No.</u>	<u>Grass</u>	<u>No.</u>	<u>Grass</u>
1	Pennstar	16	Ill. 38-17
2	Fylking	17	Sodco
3	Adelphi	18	Kenblue
4	Prato	19	Arboretum
5	S-21	20	Merion
6	Windsor	21	Code 95
7	Common (S. Dak.) #1	22	Sydsport
8	Common (S. Dak.) #2	23	Baron
9	Nugget	24	Warren's A-20
10	Primo	25	Warren's A-34
11	Geary	26	Fyl+Penns+Nug
12	Delta	27	Park+Del+Newp
13	Newport	28	Windsor+Merion
14	Park	29	Merion+Delta
15	Melle	30	S. Dak. Common+Ken blue