HERITABILITY OF IN VITRO DIGESTIBILITY IN *CYNODON DACTYLON* (L.) PERS. VAR. *ARIDUS* HARLAN et de WET

by

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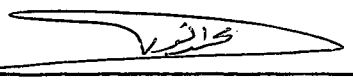
SIGNED: 

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ABSTRACT

In vitro dry matter digestibility was determined for clones of giant bermudagrass grown under different environments and for their available crosses from 1974 and 1980 samplings. Leaf/stem ratio, percent leaves per stem, and average number of leaves per stem were studied to determine their correlation with IVDMD. Frequency distributions were plotted for each cross and family of the 1980 sampling.

There were consistent differences in IVDMD among clones over a wide range of environments and degrees of growth indicating genetic control. There were also variations in IVDMD among the progenies from both 1974 and 1980 samplings. Several classes existed within each population indicating that the parents were hetrozygous for this trait.

Progeny values approached normal distributions with most means near mid-parent suggesting multiple additive gene control. However, means of high and low crosses tended to be lower than expected with modes lower than means, suggesting some partially dormant genes for low IVDMD. The differences between clones in L/S ratio, percent leaves per stem, and average number of leaves per stem were not correlated with IVDMD.

The data in this study suggest that improving DMD in giant bermudagrass is possible through breeding.

INTRODUCTION

Bermudagrass, Cynodon dactylon (L.) pers is a warm-season perennial grass which has spread around the world from its origin in tropical Africa or the Indo-Malaysian area. In tropic areas, it is most common where the rainfall is from 25 to 70 inches. In more arid areas it is found only along rivers or on irrigated land. Bermudagrass is adapted to a wide range of soils but grows best on moist, well-drained medium to heavy soils (Kneebone 1966).

Bermudagrass found its way into the U.S.A. through different introductions. Although it is probable that the first introduction to the new world was by Columbus in his second and third voyages, the first recorded introduction to the United States was in 1751 by governor Ellis of Georgia who has been credited with introducing bermudagrass to Savannah (Kneebone 1966). Heath, et al. (1978) pointed out that bermudagrass is best adapted to the states south of the line connecting the southern boundaries of Virginia and Kansas. It is considered to be the most important pasture grass in much of Southern United States (Burton 1947).

Bermudagrass is a persistent grass, after it is established, with high yield potential and consistent production from year to year. It withstands heavy grazing.

It grows over a wide range of soil pH, fertility, moisture, and salinity (up to 27,000 ppm salts) (Elder and Murphy 1961, Burton and De Vane 1975; Kneebone 1966; and Schroder 1966). High lignin and/or silica contents are associated with poor animal performance on bermudagrass (Duble, et al. 1971). The main objective of this research was to study the heritability of in vitro digestibility in giant bermudagrass.

LITERATURE REVIEW

The genus Cynodon includes nine species: C. aethiopicus clayton et Harlan, C. arcuatus J. S. Presl ex C. B. Presl. C. barberi Rang et Tad, C. dactylon (L.) pers, C. incompletus Nees, C. nlemfuensis Vandersyst, C. plectostachyus (K. Schum), C. transvaalensis Brutt-Davy and C. x. magenissi Hurcombe (Harlan, et al. 1970). The species C. arcuatus J. S. Presl ex C. B. Presl, C. dactylon var. dactylon (L.) Pers and var elegans Randle, C. incompletus var. incompletus Nees and var. hirsustus (Stent) de Wet, and C. transvaalensis Burtt-Davy are the most widely distributed (Harlan, et al. 1970).

Giant bermudagrass C. dactylon (L.) Pers. var. aridus Harlan et de Wet is diploid (2N = 18) and the probable progenator of the tetraploid (2N = 36) variety dactylon (Kneebone 1972). Harlan (1970) pointed out that giant bermudagrass is naturally distributed from South India to Palestine and the Sinai and South Africa. The variety was introduced to Hawaii and to Arizona where it is called "giant". From there it has been widely distributed by the seed trade (Harlan and de Wet 1969). Giant bermudagrass has longer internodes than those of common bermudagrass. The tillers are formed from the lateral buds which grow upward.

Large strains, with fast growing stolons sometimes may climb over shrubs three meters high. They produce a loose mat. Small strains, on the other hand, are more subdued and develop a coarse turf (Harlan, et al. 1969, and Harlan and de Wet 1970).

Bermudagrass is highly cross-pollinated (Burton 1947). Two characteristics of bermudagrass that ease the breeding program are easy vegetative propagation and a high level of self-sterility (Kneebone 1972).

New hybrids such as 'Coastal', 'Coastcross', and 'Tifton 44' bermudagrass have been developed. Coastal bermudagrass is the product of crossing an African introduction of bermudagrass on Tifton bermudagrass (Stephens 1952). Coastcross bermudagrass, on the other hand, was produced by crossing Coastal bermudagrass on Kenya 56 #14 bermudagrass. Coastcross bermudagrass yields as much per hectare as Coastal bermudagrass but has greater dry matter digestibility, apparent digestibility for all fibrous constituents, and provides faster gain per steer than Coastal bermudagrass (Utly 1971).

Brown (1976) studied the performance of five warm-season grasses including Common, Coastal, and Coastcross-1 bermudagrass. He stated that Coastcross-1 was higher than Coastal and common bermudagrass in total dry matter yield in one year but declined 18% in the next year and was

significantly higher in dry matter digestibility both years followed by Coastal and common bermudagrass.

'Tifton 44' is another hybrid bermuda, originating from a cross between Coastal bermudagrass and a bermudagrass that had survived in Berlin, Germany, for fifteen years before it was collected in 1966. Comparisons of Tifton-44 and Coastal bermudagrass showed that Tifton-44 was five to six percent more digestible than Coastal bermudagrass (Burton 1978). Utley (1978) reported that digestibility of dry matter, crude fiber, and nitrogen-free extract were greater for Tifton-44 than for Coastal in grazing trials, and the digestibility of the ether extract fraction was 4.7% greater for Coastal.

Digestibility

Harlan (1956) defined digestion as the processes which take place in the alimentary tract and which serve to break down foodstuffs into forms that can be absorbed and used by the animal body. The potential performance of animals consuming a given ration is affected by both the type and the amount of the digested nutrients available (Barnes 1973). Nelson (1975) pointed out that the nutritive value of forages for ruminants depends upon the ability of rumen micro-organisms to degrade the plant cell wall and to ferment available carbohydrates. Forage digestibility, fiber content, and their relationship to animal performance

are important aspects of improving forage quality through plant breeding (Stratton 1979).

Digestibility is an inherited character. Plants with high digestibility at any given stage tend to have relatively high digestibilities at other stages also (Julén, et al. 1966). Ross, et al. (1970) found that genotypes with superior digestibility can be selected in smooth bromegrass and that prediction of synthetic varieties having higher digestibility should be possible. Sleper, et al. (1973) indicated that improvement of whole forage and blade acid-pepsin, dry matter digestibility (APDMD) is possible through breeding in smooth bromegrass.

Burton and Monson (1972) studied the inheritance of dry matter digestibility in bermudagrass. They showed that dry matter digestibility (DMD) in bermudagrass was a heritable trait. They pointed out that the variation in DMD in F_1 hybrids involving the same parents indicated that several genes controlled the character and that the parents were hetrozygous for these genes. They concluded that digestibility was due to multiple factors with low heritability.

Methods of Digestibility Determination in Forages

Many attempts have been made in order to develop methods for the evaluation of herbage digestibility. The standard procedure to which others are compared is the total

collection method. This method requires feeding the animal for a certain period, usually several days, before starting the test in order to void the alimentary tract of other materials that might influence the results. Total feces are collected. The intake minus the amout extracted is the amount which is digested. However, this method has some problems. For example, when the digestibility of concentrate feed has to be determined, a roughage of some sort, for which digestibility had previously been determined must be fed and then the material in question added to the ration and digestibility of the complete ration is determined. The difference represents the approximate digestibility of the concentrate feed. The time required for a trial is ten days or more. During this period the forage may change, especially if it is growing rapidly, and this presents another problem (Harlan 1956).

Development of laboratory techniques for determining the digestibility of herbage made possible efficient plant breeding for this trait. For plant breeding studies, in vivo and in vitro techniques are used to evaluate the digestibility of herbage.

In Vivo Methods.

In vivo stands for inside the living body. The main idea of this method is to place in the rumen of an animal for a certain period an artificial rumen apparatus, (VIVAR

technique) or bag made of indigestible material, (Nylon Bag Technique) containing the forage sample. After a given period the apparatus is removed to determine digestion of the contents.

VIVAR Technique.

The term VIVAR stands for "in vivo artifical rumen". Fina, et al. (1962) developed this technique to study the utilization of the nutrients by rumen micro-organisms under controlled conditions inside the rumen. Mainly the apparatus consists of a stainless steel device, glass jar, or porcelain test tube. The VIVAR unit is equipped with a bacteriological membrane to control the interchange of the VIVAR and rumen contents, with a gas escape outlet. It is suspended in the rumen of a fistulated animal for a certain period (Johnson 1966, Barnes 1973).

The nylon bag technique is another in vivo method to determine the digestibility of feed. In this technique dried, weighed samples are placed in bags made of an indigestable material such as dacron, nylon, or silk and tied very well. The bags are then incubated in the rumen for certain period, and then removed to determine the digestion of the contents.

With these techniques there are some variations which would occur due to sample size and grinding size, number of

samples per trial, and location of the sample within the rumen. However, using a large sample size (10 gm), large number of samples per trial (up to forty-eight), allowing the containers to move freely in the rumen, and increasing the incubation period may reduce the variability (Johnson 1966, Barnes 1973).

The VIVAR technique may be applied for studying the effect of changing of feeding treatments on digestion and on production of volatile fatty acids (VFA). However, the nylon bag technique is useful for measuring the rate of digestion or studying the effect of various feeding treatments on the rate and extent of digestion (Barnes 1973).

In Vitro Techniques.

In contrast to in vivo, in vitro means "in glass". The main idea of this technique is to reproduce in the laboratory the reactions and conditions which normally occur and are observed within the living body. The in vitro rumen fermentation methods may be one-stage or two-stage techniques.

One-Stage In Vitro Technique.

This technique measures only the digestible fiber fraction. The technique involves an anaerobic fermentation. The sample in question is incubated under controlled conditions of temperature and pH with rumen micro-organisms in a

buffered nutrient medium (artificial saliva) for a certain period (usually forty-eight hours) and then filtered. The disappearance of dry matter represents the nutrients which are digested and converted into soluble products and volatile fatty acids (VFA) by enzymes of rumen micro-organisms (Barnes 1973).

Two-Stage Technique.

The procedure consists of two stages, the first stage a forty-eight hour fermentation by rumen micro-organisms, followed by a second stage involving solubilization by hydrochloric acid and pepsin of the residue left from the first stage. The first stage simulates reactions that take place in the rumen with breakdown of feed stuffs by microbial enzymes. The second stage simulates reactions that take place in the abomasum in breakdown of feed stuff and microbial protein in ruminant animals (Tilley and Terry 1963).

Van Soest, et al. (1966) modified the two stage technique by using a neutral detergent solution (30 gm sodium lauryl sulfate, 4.56 gm anhydrous disodium phosphate, 6.81 gm sodium tetraborate dehydrate, 10 ml ethyl cellosolve dissolved in one liter distilled water) in the second stage instead of hydrochloric acid and pepsin. The Tilley and Terry procedure gives an estimation of the apparent digestibility while the Van Soest procedure gives an estimation of the

true digestibility because with the neutral detergent solution a greater amount of dry matter is solubilized. This has been attributed to the solubilization of bacterial cell walls as well as other endogenous products.

A more recent method used for determining forage quality is infrared reflectance spectroscopy. Norris, et al. (1976) pointed out that forages can be analyzed with infrared reflectance of specific wave lengths from the sample contents. Calibrations should be made and special filters should be used for different feeds.

Shenk, et al. (1979) pointed out that plant species, particle size, and the manner in which the sample was preserved (field cured, oven dried, freeze dried, or ensiled) are the factors that affect prediction. They added that the key for prediction is the selection of wave length. Adjustments and new applications for this technology are still under investigation.

For investigations such as in plant breeding where differences among herbage samples are important, it may be preferable to use in vitro results because the precision of the results is affected only by the random errors of the method itself. Therefore, direct comparisons among samples that are closely similar in their digestibilities will be possible (Barnes 1973).

Factors Affecting Digestibility in Forages.

When forages are grown under similar conditions and cut at the same stage of growth, the primary factor governing the differences in digestibility of herbage is the genotype. Histological studies of stems and leaves after digestion show that the low digestibility of different genotypes is due largely to greater lignification of cell walls. Klock, et al. (1975) reported that poorly digestible digitgrass (Digitaria decumbens Stent) genotypes showed more secondary wall thickening in nodal regions of the stem than did those genotypes with high digestion.

Hanna, et al. (1973) examined the anatomical components of some forage leaves, including bermudagrass, after in vitro digestion. They found that the cuticle, trichomes, xylem fiber, and bundle sheathes were the only components that were not digested. They added that their observations showed that arrangement and compaction of mesophyll cells, size of intercellular spaces, and cell wall thickness affected the rate and digestion sequence of anatomical components. They suggested that maximizing digestibility in forage is possible through breeding for certain cell arrangements.

Akin, et al. (1974) compared degradation of two cultivars of bermudagrass, 'Coastal' and 'Coastcross', and 'Pensacola' bahiagrass (Paspalum notatum Flugge) by rumen

micro-organisms revealed by scanning electron microscopy. They concluded that improving the digestibility of these forages was possible by plant breeding to reduce the micro-anatomical hindrances to digestibility or by processing techniques to modify structural limitations to digestibility in the cell wall.

Hanna, et al. (1976), however, pointed out that there were no histological differences in either stems or leaves between high and low quality bermudagrass of the same age. The higher in vitro dry matter digestiblity (IVDMD) of high quality bermudagrass appeared to be due to a large soluble fraction and more digestible cell walls.

It has been reported that the differences in the digestibility among forage cultivars are due to variations in their chemical compositions. The chemical components which are suggested to be mostly responsible for the differences in digestibility are crude protein, soluble carbohydrates, fiber, and lignin. In vitro and in vivo digestibilities are correlated positively to crude protein and soluble carbohydrate levels and negatively to fiber and lignin contents (Dent and Aldrich, 1966; Arroyo-Aguilú, et al. 1975; Marum, et al. 1979; Webster, et al. 1965; and Barton, et al. 1976).

Management is an important factor that influences the yield and quality of forages. The amount of nitrogen

fertilizer application is one of the management factors that can affect the quality of grasses.

Burton and De Vane (1957) studied the effect of nitrogen fertilizer, applied from different sources, on the yield and chemical composition of bermudagrass hay. They stated that applying 448 kg/ha (400 lbs./acre) of nitrogen annually increased the protein content from 7% in unfertilized hay to 13%, increased the fiber, and reduced the ash and nitrogen-free extract, and had no significant effect upon the fat, calcium, phosphorus, and potassium content of the hay.

Webster, et al. (1965) stated that the rate of nitrogen fertilization had little or no effect upon the percent digestibility of dry-matter of bermudagrass. Their data showed an inverse relationship between lignin content and dry-matter digestiblity. Miller, et al. (1961) found that increasing nitrogen fertilizer up to 448 kilograms per hectare increased the nitrogen content of Coastal and common bermudagrass. Moreover, Binnie, et al. (1974) reported that increasing the level of nitrogen applied increased the crude protein content from 11.8 percent to 13 percent in Italian ryegrass (Lolium multiflorum).

Cutting frequency is another management factor that influences the quality of bermudagrass. Prine and Burton

(1956) concluded that hay yield was increased and protein percentage was decreased by increasing the length of cutting interval.

Burton, et al. (1963) stated that increasing the length of cutting interval from three to twenty-four weeks caused a drop in the percentage of crude protein and increased the percentage of crude fiber. Moreover, Burton, et al. (1967) found that forage from 2-, 3-, 4-, and 6-week cutting intervals differed significantly in crude protein content and nylon bag dry-matter digestibility of bermudagrass, and they decreased as age of forage increased. They also found that there were no significant interactions between genotype and age of forage.

Murdock, et al. (1961) studied the relationships of date of cutting, stage of maturity, and digestibility of orchardgrass and they reported that the most rapid decline in dry matter digestibility (DMD) occurred while the grass was advancing from booting stage to heading stage.

MATERIALS AND METHODS

This study was conducted at the Casa Grande Highway and Campbell Avenue Farms and in the animal nutrition laboratories of The University of Arizona Campus, Tucson, Arizona.

Plant Materials.

Six clones of giant bermudagrass from varied origins were chosen for study. The clones were: Yakima, I-77-1, OP_2, OP_1, B442, and B445 (Table 1). The progenies involved in the study (Table 2) were produced from crosses made by Lira (1974) at Tucson, using mutual bagging and by space isolated pairing at the Yuma Agricultural Experiment Station.

In July 1979, the in vitro dry matter digestibility (IVDMD) was determined for forage harvested from special plants of the clones grown on the Casa Grande Highway Farm plus clippings from all but I-77-1 harvested by Lira (1974) from potted plants grown in a growth chamber in 1974. The two sample series were tested together in the same run under the same conditions. Forage samples from progeny of Lira's reciprocal crosses (Table 3) were also analyzed in December 1979. Populations ranged from two to ten plants in each reciprocal cross.

Table 1. Identification and origin of giant bermudagrass clones chosen for this study.

Clone	Variety*	Origin
OP_1	afghanicus × aridus	open-pollination seedling from B442, Arizona
Yakima	aridus	Yakima, Washington
B442	afganicus	Herat, Aghanistan (P.I. 223129)
OP_2	afghanicus × aridus	open-pollination seedling from B442, Arizona
I-77-1	aridus	unknown, seedling
B445	aridus	P.I. 291616 (South Africa)

*Using classification of Harlan and de Wet (1969).

Forage from dormant parents and single seedlings from each progeny (Table 2) was harvested March 1980 at the Casa Grande Highway Farm and analyzed for IVDMD.

Forage from parents and all available progenies except B442 × Yakima (Table 2) was harvested May 1980 and parents plus five randomly selected seedlings of each progeny were analyzed for IVDMD.

The progeny of the cross B442 × Yakima, which was omitted in the May 1980 harvest, plus the parents were harvested in July 1980 and analyzed for IVDMD along with the remaining samples of May 1980.

Table 2. Parental clones and their crossed progenies grown at Casa Grande Highway Farm.

Parents	Progenies	Number Available
OP_1	$OP_1 \times OP_2$	7
Yakima	$OP_2 \times OP_1$	9
B442	Yakima $\times$ OP_1	5
OP_2	$OP_1 \times$ Yakima	14
I-77-1	$OP_1 \times$ B442	7
	B442 $\times$ OP_1	7
	Yakima $\times$ OP_2	7
	$OP_2 \times$ Yakima	10
	I-77-1 $\times$ Yakima	6
	Yakima $\times$ I-77-1	7
	I-77-1 $\times$ B442	7
	B442 $\times$ I-77-1	7
	B442 $\times$ Yakima	21

Procedures for IVDMD Analysis.

All collected samples were first dried in a light bulb dryer (Figure 1) and then ground through a Wiley mill with a 1 mm screen.

Two-1 g portions from each sample were then further dried in a vacuum oven at 100 C and 710 mm Hg. The percent dry matter was determined by using the formula.

Table 3. Parental clones and their crossed progenies from Lira study for which clippings were available for analysis.

Parent	Progenies	Number of progeny plants available
OP_1	$OP_1 \times OP_2$	7
Yakima	$OP_2 \times OP_1$	6
B442	B445 × OP_1	8
B445	Yakima × OP_1	2
OP_2	Yakima × B442	2
	Yakima × B445	10
	B445 × Yakima	10
	OP_2 × B445	4
	B445 × OP_2	10
	B445 × B442	7
	B442 × B445	3

$$\% \text{ dry matter} = \frac{\text{dry sample weight}}{\text{sample weight}} \times 100.$$

A modified Tilley and Terry (1963) technique was utilized to determine the IVDMD. McDougal's saliva buffer (McDougal 1948) was prepared by dissolving 9.8 g sodium bicarbonate ($NaHCO_3$), 9.3 g sodium phosphate ($Na_2HP_4.12H_2O$) or 5.1 g (Na_2HPO_4), 0.57 g. potassium chloride (KCl), 0.47 g sodium chloride (NaCl), and 0.12 g ($MgSO_4.7H_2O$) in one liter of

Figure 1. Convection dryer heated by light bulbs.

distilled water. Calcium chloride ($CaCl_2.2H_2O$) (.056) was added after the other compounds were in solution. Hydrochloric acid (2.2N) was prepared by diluting 200ml of concentrated HCl to one liter. Urea solution was prepared by adding 2.6 g reagent-grade urea to one liter of distilled water.

On the day before the run was made, the water bath was filled and turned on to 39°C. On the day of the run, flasks containing the McDougall's saliva buffer were placed in the water bath for preheating. Carbon dioxide was bubbled through the saliva buffer for approximately thirty minutes and the pH adjusted to approximately 6.9.

Initially, 0.5 g of the ground plant material was added to a 50ml centrifuge tube (Figure 2). Urea solution (5ml) was added to each tube at about thirty minutes before adding the saliva buffer and the rumen inoculum.

Rumen fluid collections were made from a fistulated steer maintained on alfalfa hay cubes (Figure 3) approximately three hours after morning feeding. Collections were made at about thirty minutes before adding to forage samples. The rumen ingesta were strained into a preheated flask through two cheese cloth-glass wool filters. The first consisted of one layer of glass wool between four layers of cheese cloth and the second consisted of two layers of glass wool between four layers of cheese cloth. The rumen fluid

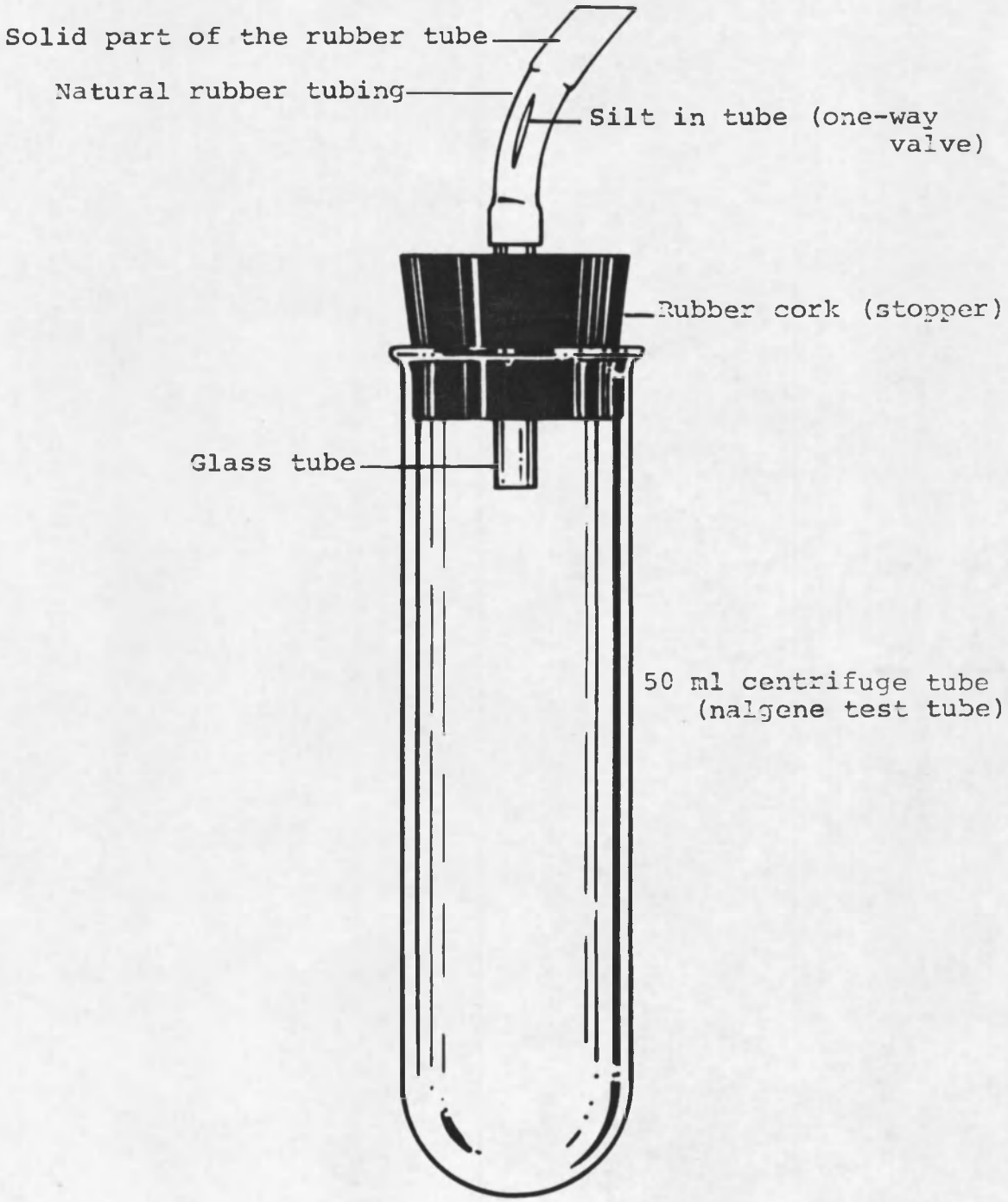

Figure 2. Schematic diagram of artificial rumen (in vitro) digestion apparatus.

Figure 3. Fistulated steer used for rumen fluid collections.

was then bubbled with CO_2 for approximately five minutes. The flasks were then covered and placed in the water bath until two distinct layers were formed. The upper layer containing bulk feed particles was discarded. Care was taken throughout the entire procedure to maintain the thermal environment (39C) necessary for maximum microbial survival. Using an automatic syringe, 20ml of the buffer solution were added to each tube that contained the 0.5g of the sample and 5.0ml of the urea solution. Tubes were then placed in the water bath. After the tubes were in the water bath, 10ml of the inoculum were added into each tube using the automatic syringe. The tubes were then flushed with CO_2 gas, capped with a one-way valve (Figure 2) and stoppered. This allowed gases to escape, while maintaining an anaerobic environment. The tubes were then incubated in the water bath at 39C for forty-eight hours, during which each tube was hand agitated at approximately twelve-hour intervals. After forty-eight hours the tubes were removed and stoppers released. Four drops of Iso-amyl alcohol were added to each tube to reduce foaming and then 2ml of 2.2N hydrochloric acid were added, to immediately stop all further microbial action. Pepsin (0.1g) was then added to each tube. The contents then mixed by gently swirling the tubes, capped with the same stoppers, and then incubated in the water bath at 39C for another forty-eight hours, during

which each tube was also hand-agitated at approximately twelve-hour intervals. Upon completion of this second stage, all tubes were removed from the water bath and the residue filtered with vacuum through glass wool in a gooch crucible. The residue with the gooch crucible was then dried in a vacuum oven. Particles added via the rumen inoculum were corrected for each sample by using a blank in every run. The percent IVDMD was calculated according to the formula:

$$\% \text{ IVDMD} = \frac{(\text{dry sample weight} - \text{dry residue weight} - \text{blank weight}) \times 100}{\text{dry sample weight}}.$$

Leaf/Stem Ratio.

Leaf/stem ratio, percent leaves, and average number of leaves per stem were calculated for the parents from ten randomly selected stems from each parent. The blades were cut carefully at the collar. The leaves and stems were dried in a vacuum oven and weighed. Leaf/stem ratio was calculated according to the formula:

$$\text{L/S ratio} = \frac{\text{dried leaf weight}}{\text{dried stem weight}}.$$

Percent leaves was calculated according to the formula:

$$\% \text{ leaves} = \frac{\text{dried leaf weight} \times 100}{\text{dried leaf weight} + \text{dried stem weight}}.$$

Analyses of variance for IVDMD were conducted for the parents from different dates, for the progenies and for each family of reciprocal crosses harvested in 1980. Least significant differences were calculated for each data source. Frequency distributions were plotted for each cross and family using three percent intervals and converting the number of individuals in each class into percent of the total population.

RESULTS AND DISCUSSION

Parents

Variances for the parent clones were significant at the .01 level in analyses made for each sampling date and in a combined analysis for the 1979 and 1980 samplings. In the combined analysis, the variance for dates was significant at the .01 level. The date by parent interaction was also significant at the .01 level. Similar analysis made with the four parents included in the Lira sampling gave similar results with the exception that the date by parent interaction was not significant. Burton, et al. (1967) also found no significant interaction between genotypes and age of forage.

Average IVDMD values from all samplings are given in Table 4. Those for OP_1 and Yakima were consistently high, OP_2 and I-77-1 consistently low. B442 and B445 had intermediate values.

OP_1 had significantly higher IVDMD values than B442 at all sampling dates while OP_2 values, although lower than those for B442 in four of five samplings, were significantly lower only twice. The values for Yakima were sometimes higher and sometimes lower than those for OP_1. Similar changes occurred with OP_2 and I-77-1. These fluctuations

Table 4. Mean IVDMD percentages of parent clones at each sampling date.

Parent	Sampling Dates					Parental Means	
	7/79	8/79	3/80*	6/80	1974**	79-80	75-79-80
OP_1	57	63	48	57	69	56	59
Yakima	59	64	44	53	63	55	57
B442	51	56	40	53	60	50	52
OP_2	45	59	38	50	59	48	50
I-77-1	49	54	39	46	--	47	--
B445	--	--	--	--	60	--	60
Sampling Means	52	59	42	52	63	51	55
LSD .05	2.0	3.2	4.7	1.6	4.9	2.5	4.4
Range	45-59	54-64	38-48	46-57	59-69	47-56	50-59

*Dormant plants and cured on the stalk.

**Plants from Lira (1974) grown in growth chamber.

in degree and to some extent order, probably explain the significant interaction. Since a combined analysis of four parents including the Lira sampling showed no significant interaction, and OP_1 and Yakima were consistently higher than OP_2 and I-77-1, regardless of sampling date, it is probable that interaction effects can generally be ignored in this evaluation.

There were real differences in quality regardless of the condition of the forage at sampling time. Forage in March 1980 was completely dormant and cured on the stalk. Material from the Lira study came from pots in a growth chamber study and was mostly leaf. Average IVDMD's were 42% and 63% respectively, yet ranges and rankings were the same (Table 4).

Consistent differences among the parents over a wide range of environments and degrees of growth indicate genetic control of digestibility. OP_1 and OP_2 are half sibs with the common female parent B442. They differed widely in IVDMD with OP_2 similar or less and OP_1 significantly higher than their mother plant.

Assuming genetic differences, the question arises, "affecting what?". One reason might be differences in leafiness. Leaf/stem ratio, percent leaves, and average number of leaves per stem sampled in August 1980 (Table 5) showed that OP_1 and OP_2, with high and low digestibilities had the highest leaf/stem ratios and percent leaves. Yakima and I-77-1, also with high and low digestibilities had the lowest leaf/stem ratios and percent leaves. Although OP_2 had fewer leaves per stem than the more digestible parents, I-77-1 with low IVDMD had leaf number similar to the high parents. B442, which was lower in IVDMD than Yakima, had relatively higher leaf/stem ratio and percent leaves. Although there

Table 5. Leaf/stem ratio, percent leaves, and average number of leaves per stem for the five cultivars sampled August 1980.

Cultivar	Leaf/stem ratio	% Leaves	Average no. leves per stem
OP_1	0.82	45	11.5
Yakima	0.47	32	11.3
B442	0.55	35	10.6
OP_2	0.85	46	9.0
I-77-1	0.38	27	11.0

were differences among parents in leaf/stem ratio and percent leaves, these differences were not correlated with IVDMD differences. The average number of leaves per stem might have had some effect. The study of Hanna, et al. (1973) on the histology of fresh forage leaves, including bermudagrass, after in vitro digestion suggested that the arrangement of the mesophyll cells, cell wall thickness, and size of intercellular spaces affected digestibility. Preliminary studies indicated that some of those cell arrangements were genetically controlled. Similar results were obtained by Klock, et al. (1975) with digitgrass. Their microscopic examinations showed that genotypes which were poorly digestible had thicker secondary cell walls in nodal regions than those with high digestibility.

Genetic differences among the parents in this study probably also have their main effect on internal rather than external factors and histological studies might show correlated differences.

Progenies

Mean values for the progenies (Table 6) showed that F_1 hybrids had IVDMD values greater than those for their parents (Table 4). They also show that average progeny values ranked similarly to average parental values whether parents were used as male or female. The highest mean IVDMD was 64 percent for the hybrids OP_1 × Yakima, OP_1 × B442, and OP_2 × OP_1. The lowest was 59 percent for the hybrids Yakima × I-77-1, I-77-1 × Yakima, and B442 × I-77-1 while the highest mean value for the parents in the June 1980 sampling was 57 percent for OP_1 (Table 4).

The higher values for the progenies were probably due to more vigorous predominantly vegetative growth associated with a younger stand. Although the parents were also grown as spaced plants in the same irrigated border they were established as large plugs a year before the progenies were established as seedlings.

Although these plants differed significantly in IVDMD under a variety of conditions, the significant differences in values between samplings show how important it

Table 6. Mean IVDMD percentages, numbers of individuals, and ranges for progenies sampled in June 1980.*

Male Parents → / Female Parents ↓	OP_1			Yakima			B442			OP_2			I-77-1			Mean as females
	Mean	# of Plants	Range	Mean	# of Plants	Range	Mean	# of Plants	Range	Mean	# of Plants	Range	Mean	# of Plants	Range	
OP_1	-	-	-	64	14	59-67	64	7	61-69	63	6	60-66	0	0	0	63.7
Yakima	61	5	57-65	-	-	-	0	0	0	61	7	57-67	59	7	55-64	60.3
B442	62	7	56-57	60	21	55-64	-	-	-	0	0	0	59	7	52-66	60.3
OP_2	64	8	62-67	63	10	60-69	0	0	0	-	-	-	0	0	0	63.5
I-77-1	0	0	0	59	6	56-64	60	7	57-64	0	0	0	-	-	-	59.5
Mean as males	62.3			61.5			62.0			62.0			59.0			

*B442 × Yakima were sampled in July 1980.

is to make comparison only at similar stages of growth and similar growth conditions. Accurate prediction of digestibility using the two-stage in vitro technique also requires a constant source of inoculum (Harton, et al. 1980). Inoculum for all the IVDMD determinations in this study came from the same steer fed a uniform diet of alfalfa cubes.

Parental and progeny IVDMD values from Tables 4 and 6 are summarized in Table 7 with expected progeny values based on parental means adjusted according to the overall parent/progeny ratio. Although progenies of high parents had relatively high values, these were lower than expected. Those from low parents were near to or somewhat higher than expected values.

Frequency Distributions of Progenies.

Individuals within each cross (Table 7) were grouped in percentiles to enable direct comparisons (Figures 4 through 10). The number of IVDMD classes ranged from three for $OP_1 \times OP_2$ to five for B442 × Yakima. The three percent interval was the average of L.S.D. values at .05 level for within population comparisons (2.98). Yakima and OP_1 from the July 1979 sampling were used as controls in each run. The average difference between laboratory duplicates was 1.21 percent for OP_1 and 1.4 percent for Yakima. Maximum spreads between runs were 2.77 for OP_1 and 0.25 for

Table 7. Expected progeny IVDMD values based on parental means adjusted according to the overall parent/progeny ratio.

Parent	% IVDMD 6-1980	% IVDMD of Progenies when parent used as			Expected Values*	
		Female	Male	Combined	Progeny	Mid-parent
OP_1	57	64	62	63	68	64
Yakima	53	62	60	61	63	63
B442	53	60	62	61	63	61
OP_2	50	64	62	63	60	62
I-77-1	46	60	59	60	55	59
mean	52	62	61	62	62	62

*Values adjusted by mean progeny/parent ratio $(\frac{62}{52})$.

Yakima. In the animal nutrition laboratory at the University of Arizona, differences of three percent or less between replicates are not considered to be significant.*

The distributions of the crosses OP_1 × Yakima and its reciprocal (Figure 4) were different, but the numbers in each cross were different and few conclusions can be drawn. The

*Personal discussion with Dr. R. S. Swingle, Animal Sciences Department, University of Arizona.

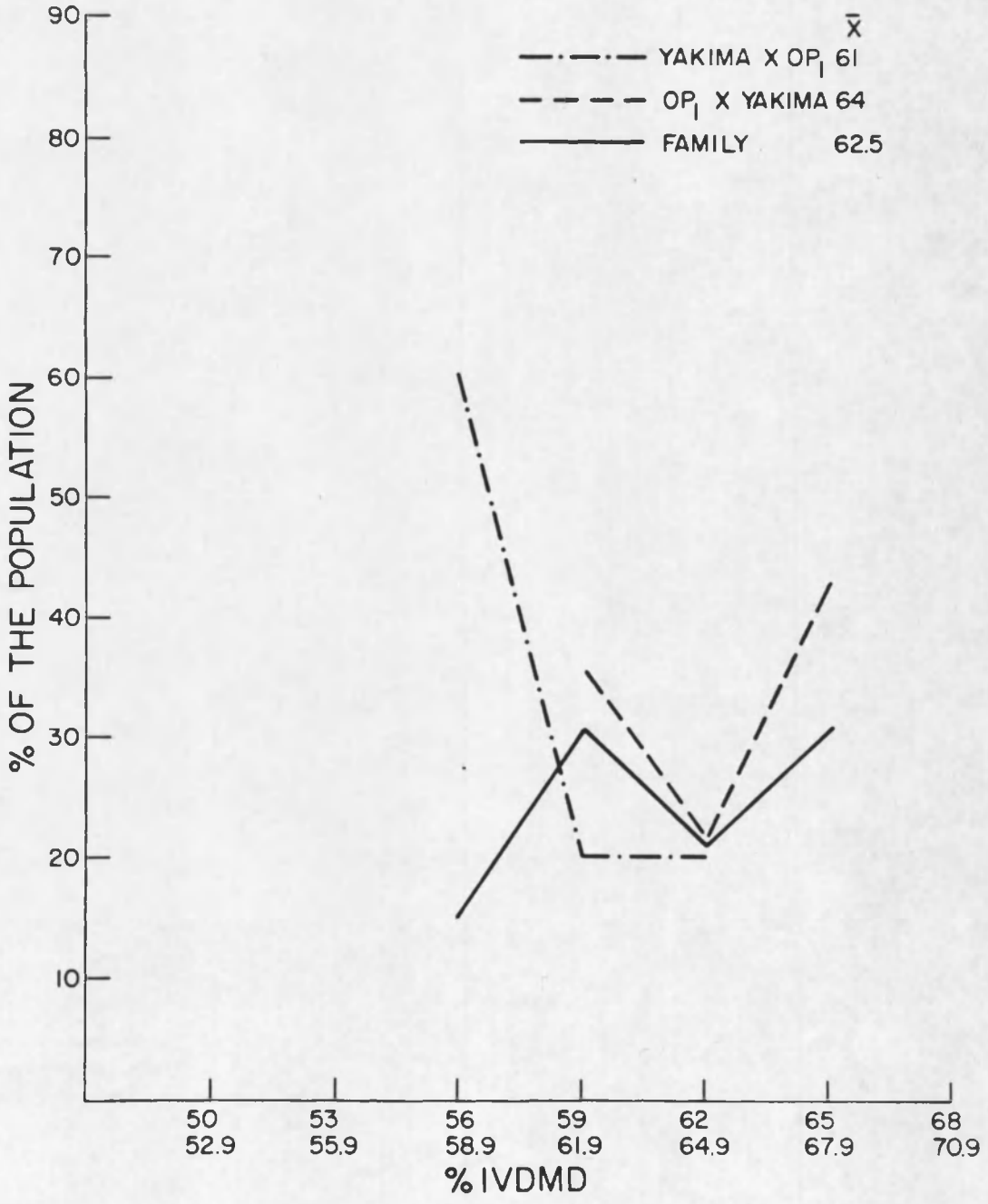

Figure 4. Frequency distributions for F_1 progenies of Yakima and OP_1.

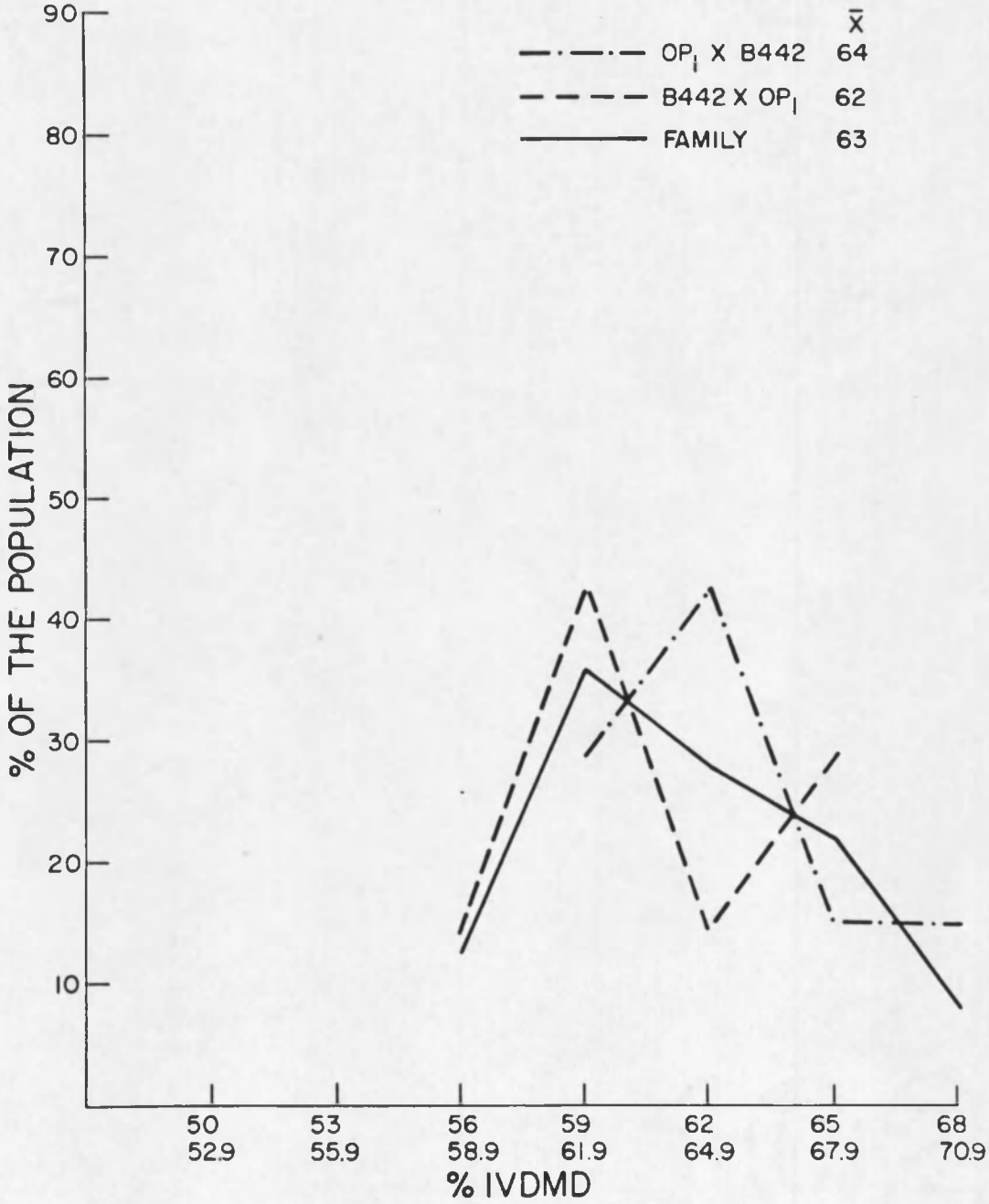

Figure 5. Frequency distributions for backcross progenies of OP_1 and B442.

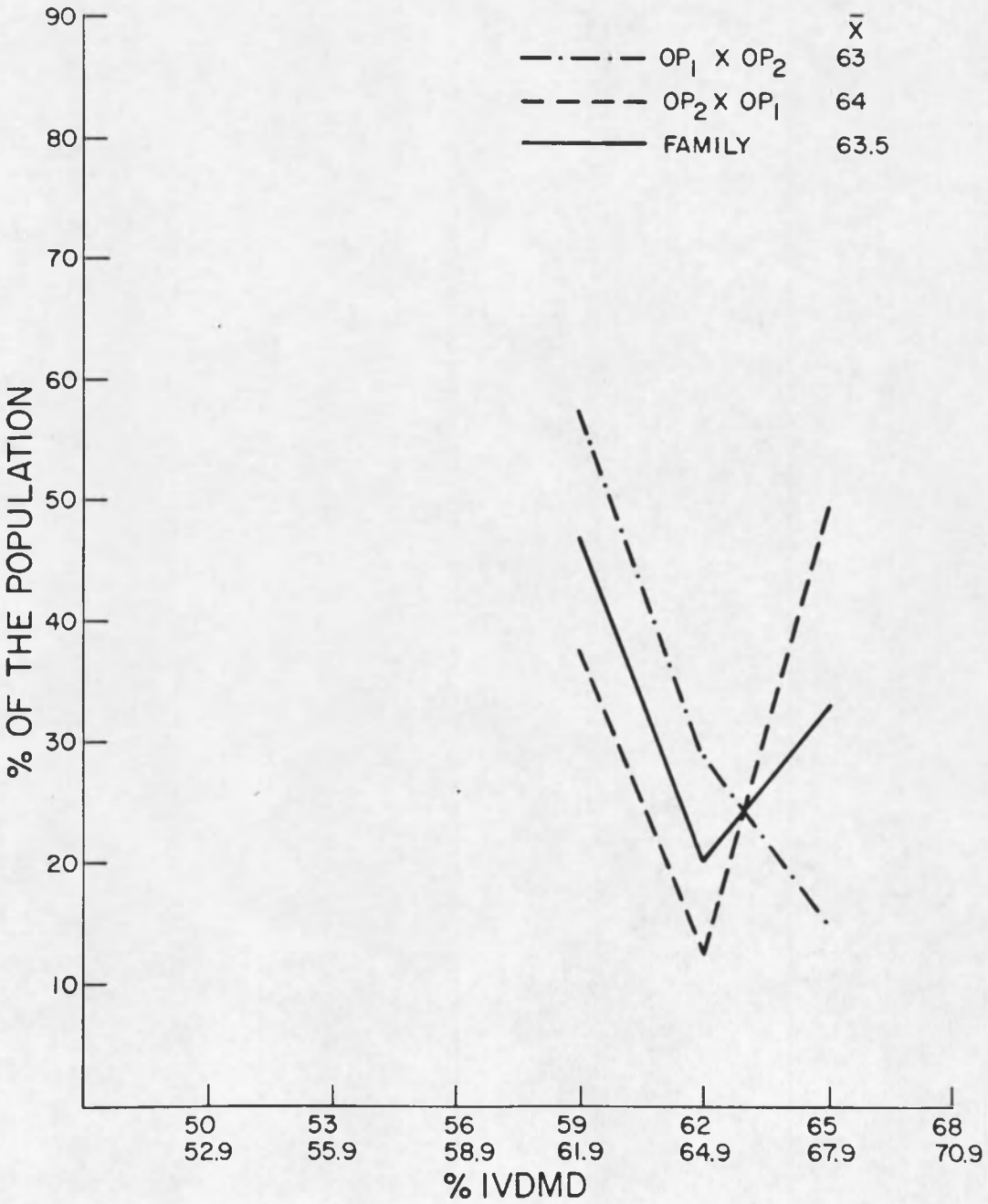

Figure 6. Frequency distributions for F_1 progenies of OP_1 and OP_2.

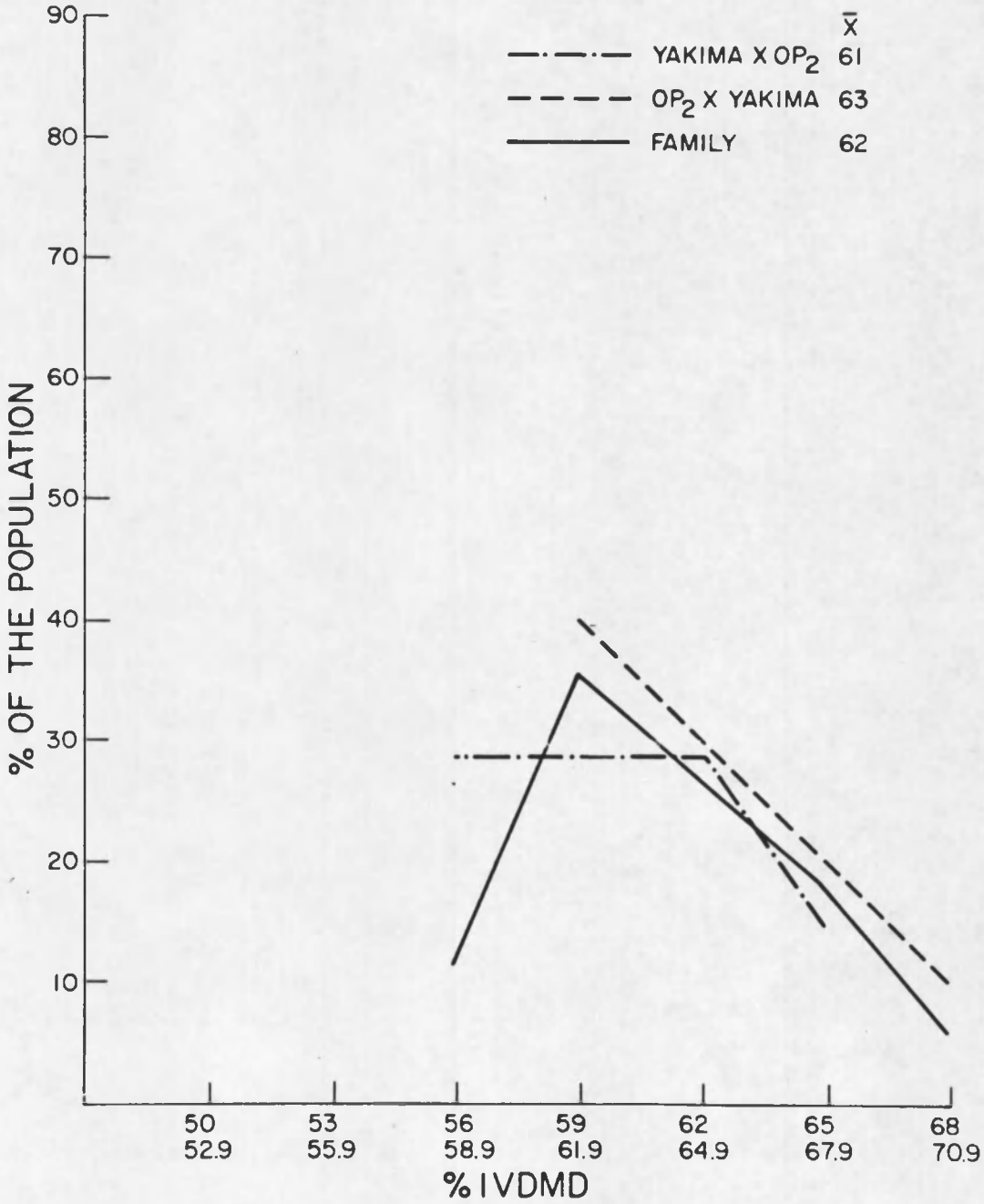

Figure 7. Frequency distributions for F_1 progenies of Yakima and OP_2.

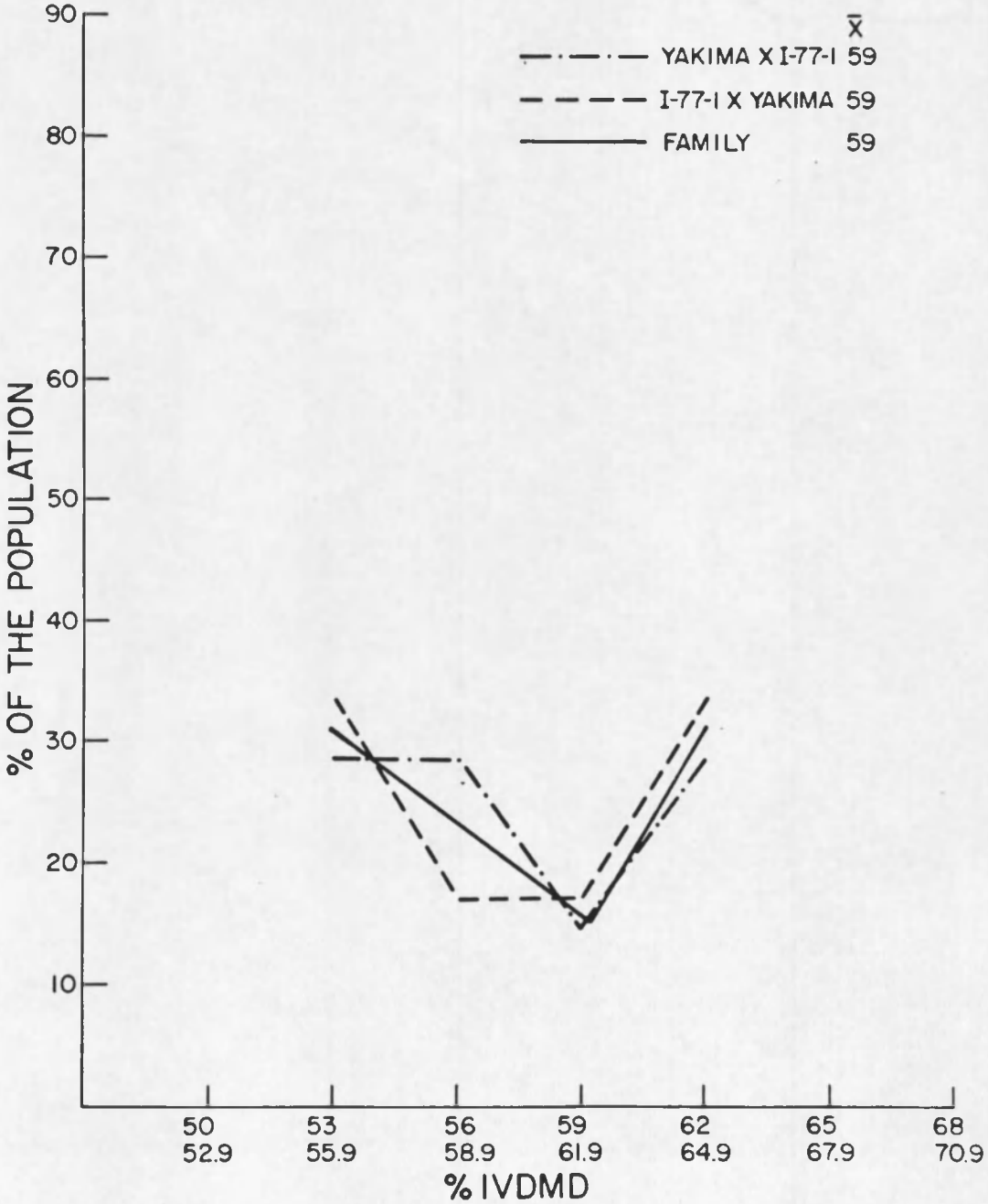

Figure 8. Frequency distributions for F_1 progenies of Yakima and I-77-1.

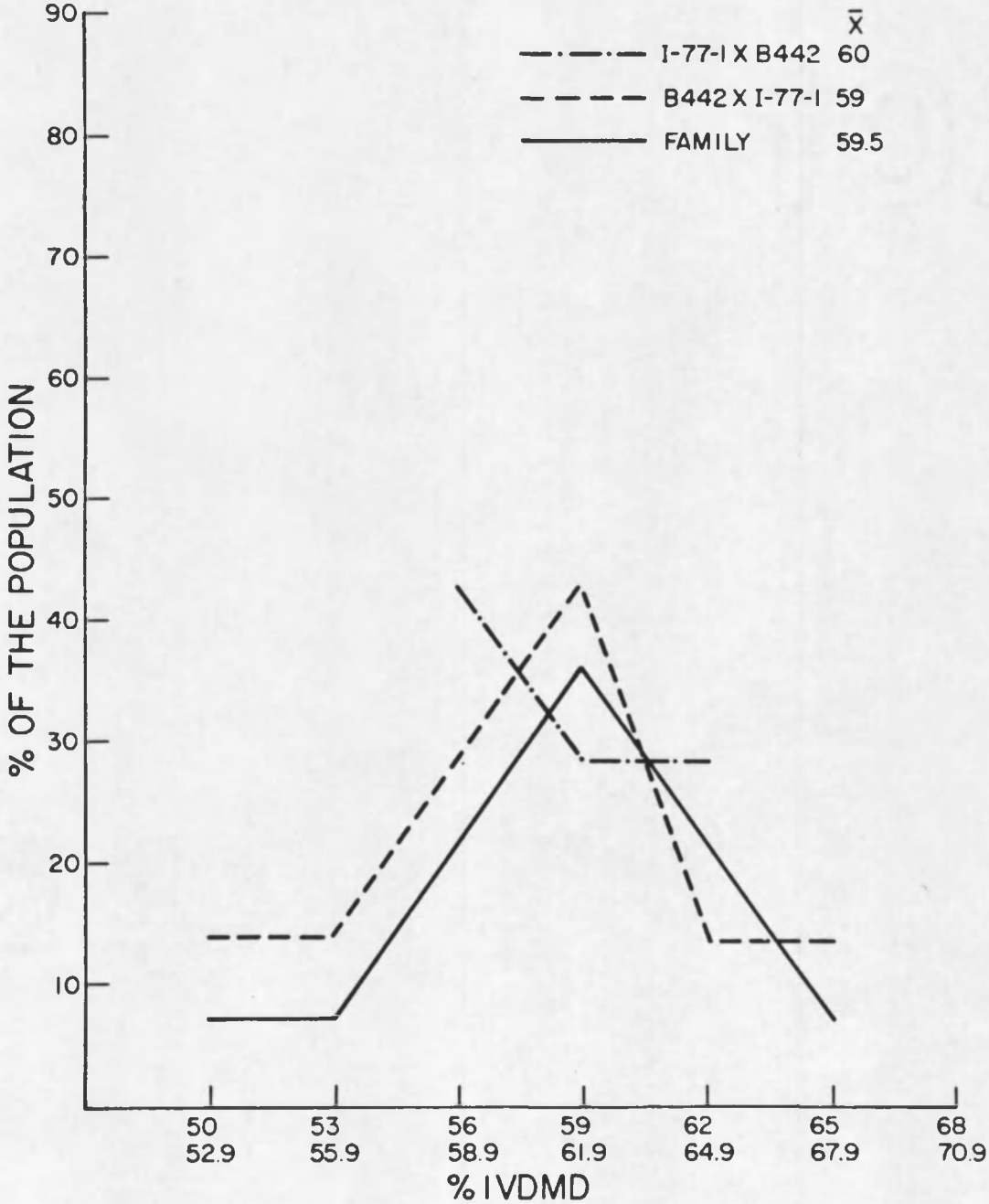

Figure 9. Frequency distributions for F_1 progenies of I-77-1 and B442.

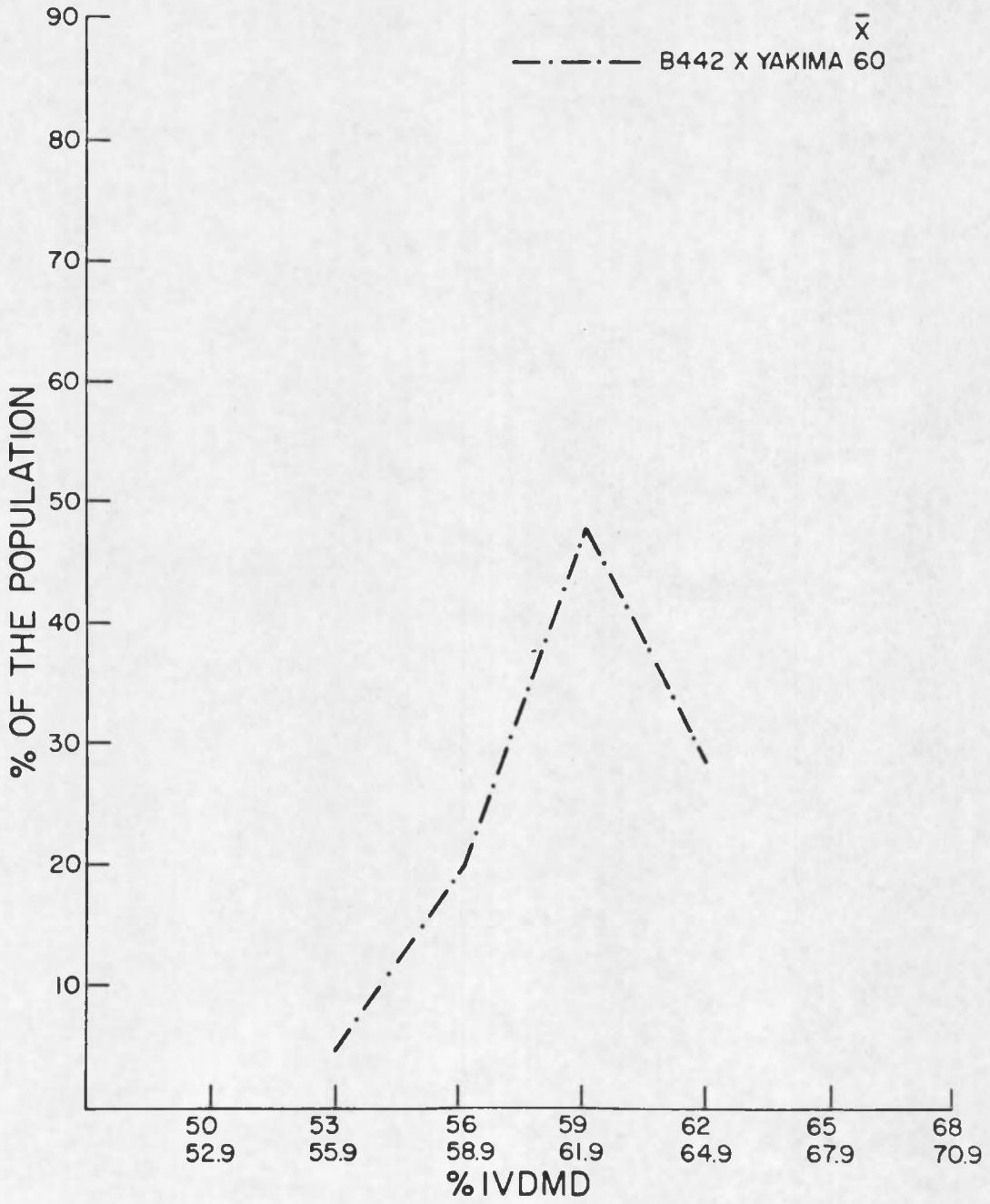

Figure 10. Frequency distributions for F_1 progenies of the cross B442 × Yakima.

distribution pattern for the combined data is probably much more representative of the cross than either reciprocal. There were four classes, each representing from 17-31% of the population.

OP_1 is an open pollination seedling from B442. Each of the reciprocal back crosses suggested normal distribution as did the family curve. The distributions were skewed to the left in all crosses with mean values higher than modal values (Figure 5). The F_1 plants from OP_1 and OP_2 (Figure 6) had IVDMD values which gave a bimodal curve with three classes. The mean values were in the second class while the mode was in the first class where forty-seven percent of the population had low digestibility. The distributions of Yakima × OP_1 and its reciprocal included four classes (Figure 7). The curve for the combined data approached normal distribution but was skewed to the left with five classes. The mode was lower than the mean.

The F_1 IVDMD values for I-77-1 and Yakima were bimodally distributed with four classes (Figure 8). This is a similar distribution to that from OP_1 by OP_2, also a high by law cross. The individuals of the cross B442 × I-77-1 were distributed normally but not those of the reciprocal (Figure 9). The family distribution was normal with six classes. The overall mean value and the modal values were in the same class. However, among the progenies of these

crosses there were some individuals with very low digestibility. The distribution of the cross B442 × Yakima (Figure 10), for which the reciprocal was not available, suggested a normal distribution. The mean and mode were of the same class.

For almost all the families, the modal values were near or in the low digestibility classes, even though there were some individuals with high digestibility. This, plus mean progeny values lower than expected from high parents and near expectation from low parents suggests partial dominance for low digestibility. Similarly, progeny means lower than mid-parent values (Table 7), lower than those expected from high parents, and near expectation from those from low parents also suggest partial dominance for low digestibility.

The cross $OP_1 \times OP_2$ and its reciprocal gave high mean values (Table 6), even though OP_2 was a poorly digestible parent. The bimodal curves for the family distribution (Figure 6) suggest that OP_1 and OP_2 had major genes for high and low digestibility with more low digestibility genes in OP_2. The bimodal distribution of the F_1 IVDMD values for I-77-1 and Yakima also suggest that I-77-1 and Yakima had major genes for low and high digestibility with I-77-1 having more genes for low digestibility. However, the curves

of Yakima × OP_2 and Yakima × I-77-1 indicate that the low digestibility genes of OP_2 are not the same as I-77-1.

The distributions of F_1 IVDMD values for Yakima and OP_1 (Figure 4) did not give a consistent picture. The modal values and mean in the cross, however, were in the higher digestibility class indicating that these two parents have many positive genes for high digestibility. Moreover, the overall mean values were high for all the crosses in which OP_1 is involved (Table 6) which, in turn, indicates that OP_1 has positive genes for high digestibility. This case was true also with the back crosses of OP_1 and B442. The skewness to the left in the family distribution of this cross indicates dominance for low digestibility (Figure 5) as does that for Yakima and OP_2 (Figure 7). Having about fifty-three percent of the population with high digestibility (more than sixty-two percent IVDMD) indicates that OP_2 had some genes for high digestibility but these genes were recessive and segregated in the progeny. The normal distribution of the family IVDMD for I-77-1 and B442 as well as the other normal curves indicate the multigenic genetic control of IVDMD in bermudagrass.

Values from the Lira samples (1974) also indicate partial dominance for low digestibility (Table 8). Crosses involving OP_1 and Yakima tended to give progeny mean values lower than mid-parent values. B445 crossed with OP_2 and

Table 8. Mean IVDMD percentage from the 1974 sampling for parents and progenies with numbers and range of values for each progeny.

Parent	Mean IVDMD	Cross	Mean IVDMD	No. of Plants	IVDMD Range
OP_1	69	OP_1 OP_2	58	7	53-60
Yakima	63	OP_2 OP_1	61	6	60-63
B442	60	B445 OP_1	62	8	56-67
B445	60	Yakima OP_1	58	2	54-63
OP_2	59	Yakima B442	55	2	53-57
		Yakima B445	59	9	54-61
		B445 Yakima	62	10	58-65
		OP_2 B445	50	4	55-63
		B445 OP_2	60	10	54-64
		B445 B442	52	7	45-59
		B442 B445	60	3	59-61

with Yakima gave progenies with mean values near the mid-parent indicating no dominance for genes with this parent.

Burton and Monson (1972) suggested that multiple genes with additive effects but no dominance were involved in IVDMD. Data from this study would indicate both additive and dominance effects with many genes involved. The effects could be on the cell wall thickening or lignification and this in turn would have a negative effect on IVDMD. Since

the progenies of all crosses had some individuals that were high and others that were low for IVDMD, all of the parents were heterozygotes.

Since IVDMD in bermudagrass is genetically controlled, improving IVDMD is possible through breeding for this character. The data in this study indicated that the progenies derived from crossing two highly digestible parents were better than those derived from crossing high by low or low by high and these were better than those derived from crossing two poorly digestible parents. The data in this study indicate that selection for high individuals presupposes a concentration of favorable additive genes and some limit on unfavorable dominants. Progeny testing provides a way to choose and develop parents homozygous for favorable recessives and with the high combining ability that multiple favorable additive genes would provide.

CONCLUSION

There were significant variations in IVDMD among the parents in a wide range of environments and degrees of growth and these variations were consistent, indicating genetic control. The variation in IVDMD among the progenies of those parents and the existence of several classes within each population indicate that the parents were heterozygous. Normal or near normal progeny distributions suggest control by multiple additive genes. Skewness to the low side of the progeny distribution and mean progeny values less than expected suggest partial dominance for low digestibility. The leafiness data showed that apparently neither leaf/stem ratio nor percent leaves affected IVDMD differences. The differences among parents were apparently internal rather than external suggesting the need for histological studies.

The data suggest that improving DMD in giant bermudagrass is possible through breeding. The potential for improved animal production from an increase in digestibility without yield change is the same or possibly better than the potential for an equivalent percentage increase in yield. Burton, et al. (1967) estimated that an increase of approximately 10% in digestibility would give a 30% increase in animal gain.

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