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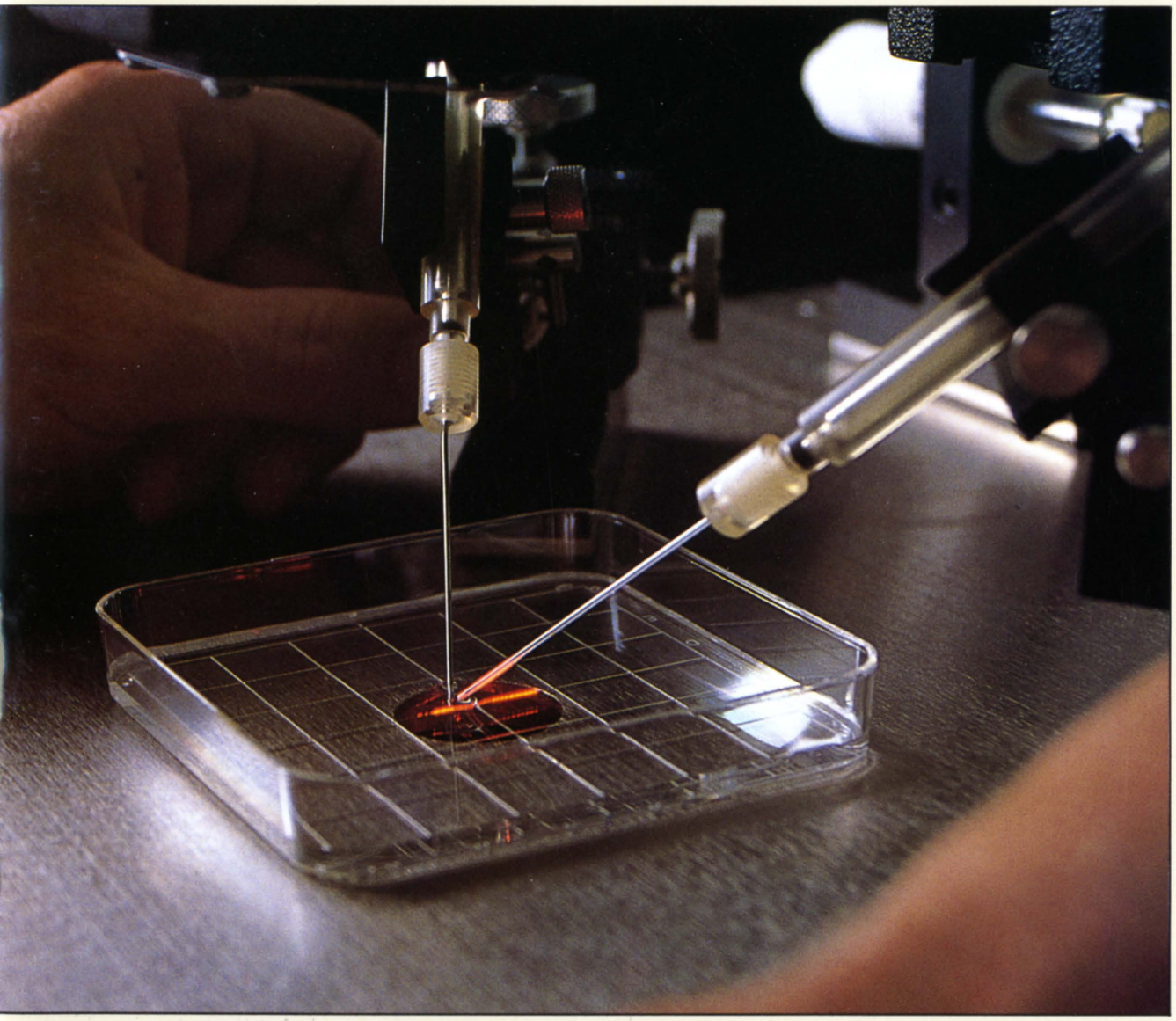

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UTAH SCIENCE

UTAH AGRICULTURAL EXPERIMENT STATION VOLUME 49 SPRING 1988 NUMBER 1

THE BLUE ORCHARD BEE: AN ALTERNATIVE POLLINATOR OF APPLES

P. TORCHIO

Apples are one of many commercial crops requiring cross-pollination (transfer of pollen between flowers of compatible cultivars) to produce fruit. Cross-pollination requires that two or more apple cultivars be planted in each orchard; honey bees have usually been used to transfer pollen from flower to flower.

Successful pollination of apples depends on a somewhat unique set of physiological and floral characteristics, any one of which can dramatically influence the efficiency of pollination. For example, pollination can be adversely affected because trees flower early in the year during cool-wet periods, and each tree produces massive numbers of flowers during a short period. The floral anatomy of cultivars can also influence foraging behavior of honey bees because the flowering periods of varieties or cultivars in an orchard overlap, and cross-pollination can occur only during the overlap period.

Pollination is also affected by the fact that larger flowers on each tree ("king bloom") appear before four smaller flowers ("queen bloom") that surround the king blossom. Every flower has five female structures (styles), each of which ends at a stigmatic surface that is receptive to pollen (the male component) for only a short time prior to petal fall.

Thus, ideal pollination in commercial orchards requires that the pollinator pay multiple visits to apple flowers to ensure that pollen is transferred to each stigmatic surface of all blossoming cultivars. When that occurs, maximum numbers of king florets develop into fruit. King blossoms have thicker pedicels that resist frost damage and also permit development of larger apples. Hormones released during seed development also control the market quality of ripened fruit.

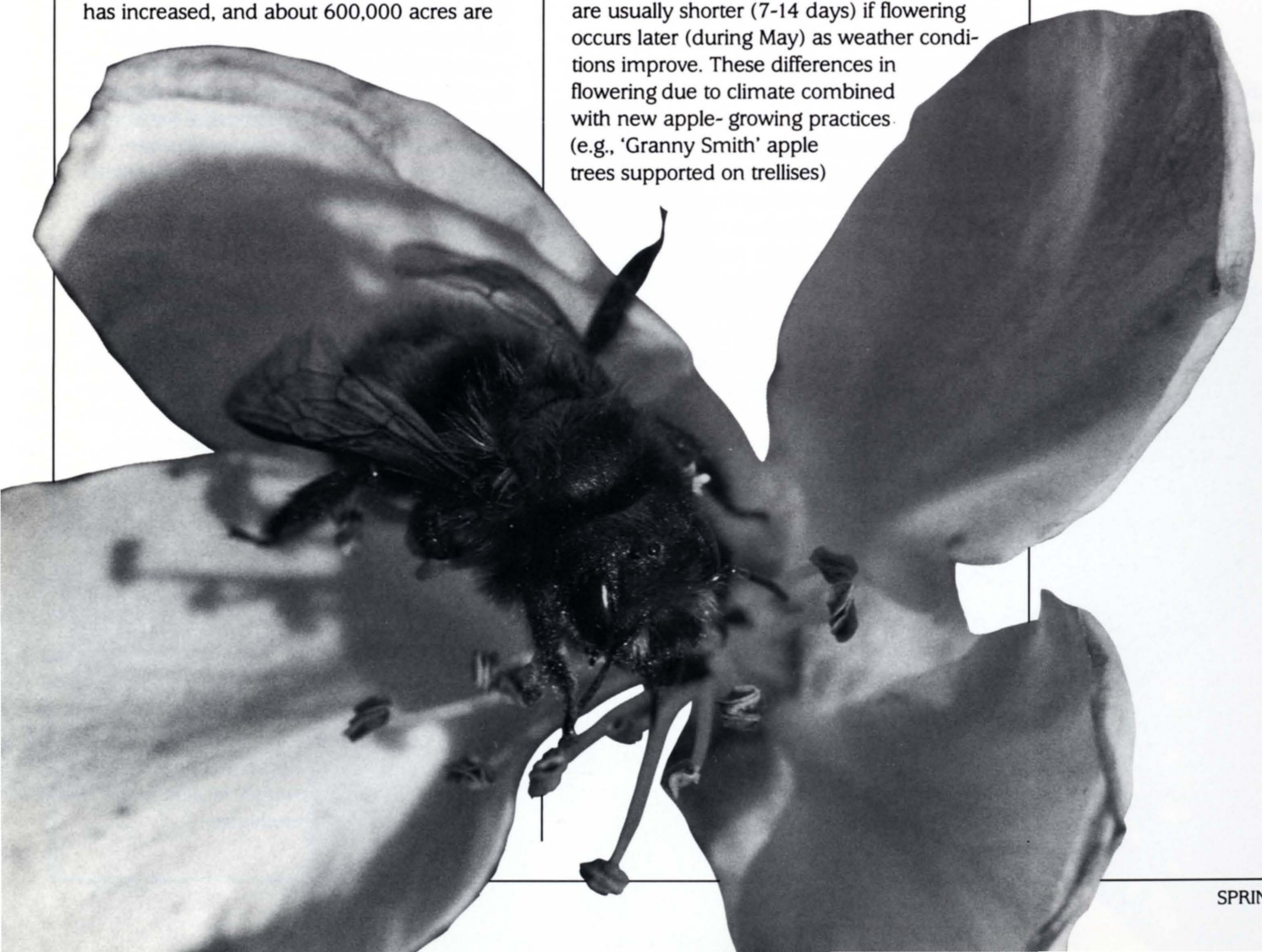
Some growers believe that partial pollination of apple trees will result in an adequate crop. These growers remove honey bee colonies during periods of peak bloom. Most growers, however, leave honey bee colonies throughout blossoming periods, a practice which increases the probability that most flowers of all cultivars will be fully crosspollinated and which results in maximum yields of large-sized,

properly formed, high-quality fruit year after year. The costs associated with thinning developing fruit following maximized pollination are outweighed by the returns associated with maximum yields of quality fruit.

The pollination of commercial apple crops in the United States has been complicated by several important changes during the last 30 years. Dramatic improvements in storage technology now mean that fresh apples are available year-round. Demand for fresh fruit has increased, and about 600,000 acres are now devoted to apple orchards. New and exotic varieties of apples introduced into U.S. markets have been readily accepted by consumers. As a result, large apple orchards in a number of climatic zones within the United States now contain numerous cultivars. Apple trees in warmer zones come into flower from March to April and trees in cooler zones flower from April to May. Early flowering (March), which normally occurs during cooler and wetter conditions, can extend bloom periods to 28 days or more; bloom periods are usually shorter (7-14 days) if flowering occurs later (during May) as weather conditions improve. These differences in flowering due to climate combined with new apple-growing practices (e.g., 'Granny Smith' apple trees supported on trellises)

Ideally, a pollinator should visit apple flowers several times to make sure pollen is transferred to all blossoming cultivars.

A female blue orchard bee on an apple blossom.



can affect the pollination efficacy of honey bees. These factors may also explain why yields per acre have decreased as acreage devoted to apples has increased.

There has also been a reduction in the number of honey bee colonies available for pollination during the last 30 years. The recent introduction of the parasitic tracheal and Varroa mites into U.S. honey bee colonies and the likelihood that Africanized bees will enter through our southern borders almost guarantee that there will be a further reduction in the number of honey bee colonies in this country. If so, there may be a shortage of bees available to pollinate agricultural crops grown in Utah and the United States. This would reduce yields.

Honey bee research is an integral part of federal research to correct these problems. The U.S. Department of Agriculture supports the Bee Biology and Systematics Laboratory on the USU campus is devoted to the study of non-honey bee species. One research program at this laboratory concerns the feasibility of developing non-honey bee species as effective pollinators of targeted crops. Orchard crops became a focus of this research in 1970. Since then at least one non- honey bee species, *Osmia lignaria propinqua* (blue orchard bee), has been fully developed as an alternative pollinator of orchard crops (including apples).

Most of the field research concerning use of the blue orchard bee as a pollinator of orchard crops was conducted in northern Utah. This article describes this research.

A glass tube used to study in-nest behavior.

Early Studies

The study of alternative pollinators for orchard crops initially involved a survey of wild and domesticated orchard crop species to determine which bees were attracted to flowers of those plants. Only one of the bee species, the blue orchard bee, was present in large numbers at all sites. Following a review of literature concerning *Osmia lignaria*, experiments were designed to determine the size of natural populations and the suitability of man-made nest materials, and to identify the nest materials and hole sizes that were most attractive to nesting populations of this bee.

During a 5-year field study, we learned that there were large populations of blue orchard bees in the Intermountain states, as indicated by nesting success in man-made nest materials. Wood in which holes 7 mm in diameter and 15-16 cm deep had been drilled provided the best nesting system. Storing the nests in temperature-controlled cabinets made it possible to control bee emergence by manipulating winter temperatures. Flight periods, nest architecture, fecundity, rates of parasitism, and other biological features of blue orchard bees were also determined.

Biology of the Blue Orchard Bee

Individual bees were marked and observed in the field to determine foraging behavior. We also observed in-nest behavior in boxes fitted with glass-tubes located in the field and greenhouse.

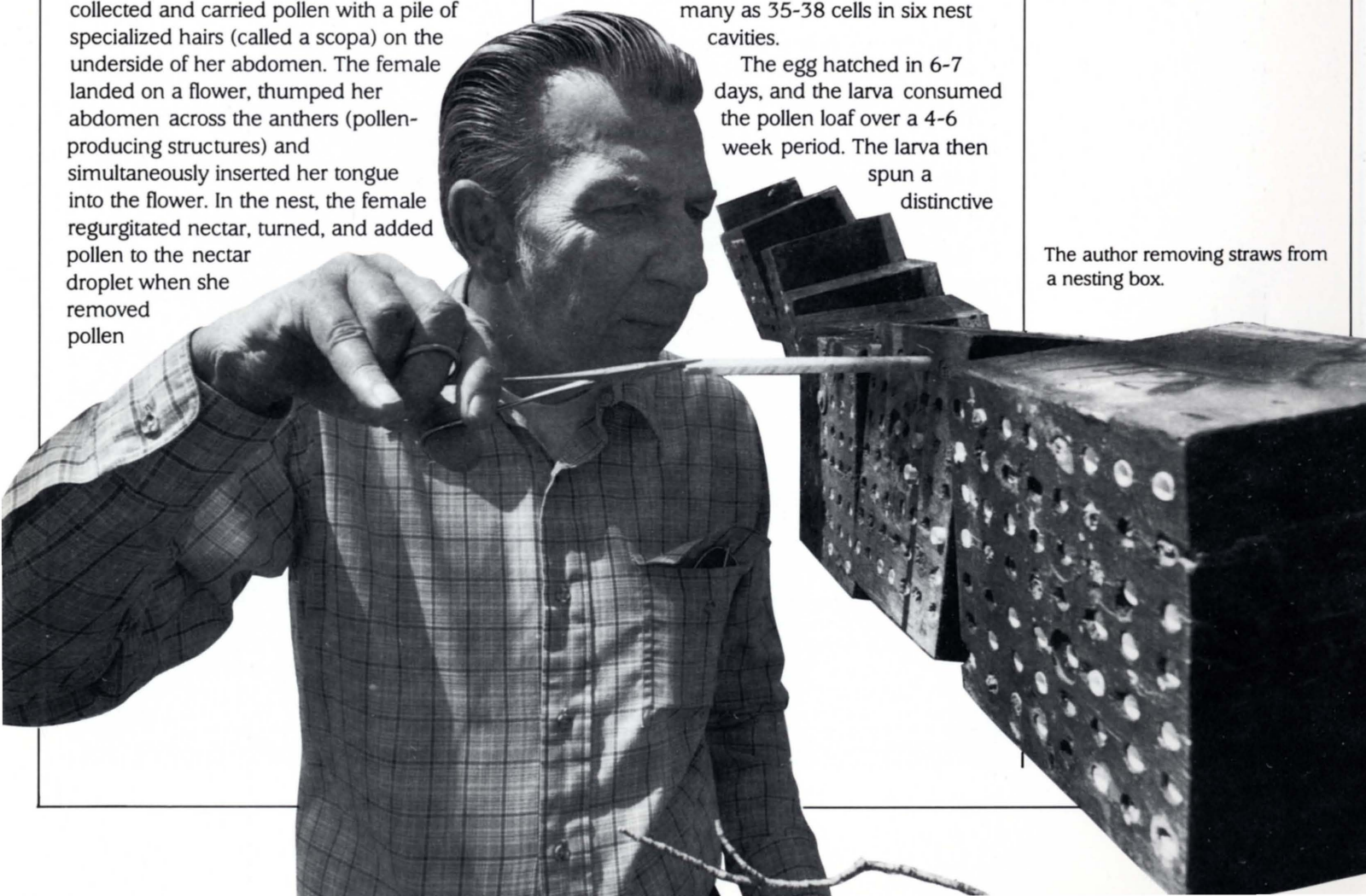
Researchers found large populations of blue orchard bees in the Intermountain states.

Males emerged in synchrony with the initiation of apricot bloom, and females emerged 3-14 days afterwards. Mating occurred only on the day of female emergence, and mated females spent the second day of flight searching for and establishing a nest in existing cavities. Nesting was initiated on the third day of flight when the female carried mud into the cavity, which she used to construct a partition across the base of the cavity. She then provisioned the first cell with pollen wetted with nectar until a pollen loaf covered all or most of the outer surface of the mud partition.

Unlike the honey bee that carries wetted pollen on its hind legs, the blue orchard bee collected and carried pollen with a pile of specialized hairs (called a scopa) on the underside of her abdomen. The female landed on a flower, thumped her abdomen across the anthers (pollen-producing structures) and simultaneously inserted her tongue into the flower. In the nest, the female regurgitated nectar, turned, and added pollen to the nectar droplet when she removed pollen grains from her scopa by repeatedly scraping her hind legs. The female bee made 14 to 35 pollen-collecting trips to provision one cell before laying an egg on the surface of the completed pollen loaf.

She closed the cell by constructing another mud partition across the diameter of the nest tube in front of the completed provisions. She then constructed a linear series of cells and covered the nest entrance with a nest plug (also composed of mud). The female established another nest in a second cavity and could construct about one cell per day until she died. Nesting activities continued for 5-6 weeks, during which the female produced as many as 35-38 cells in six nest cavities.

The egg hatched in 6-7 days, and the larva consumed the pollen loaf over a 4-6 week period. The larva then spun a distinctive



The author removing straws from a nesting box.

Immatures also developed normally in orchards, and their nesting behavior in the orchard was similar to that observed in natural environments.

cocoon and soon molted into a pupa. The pupa darkened over a 2-week period and then molted into the adult form that overwintered in the cocoon. Bees emerged the following spring in response to rising temperatures.

Orchard Studies

The first orchard study began in 1974 when bees were introduced into a local semi-abandoned apple-plum orchard. A study of this small population showed that these bees nested successfully in man-made materials. Females visited both apple and plum flowers and their provisions were composed mostly of apple-plum pollen. Immatures also developed normally in orchards, and their nesting behavior in the orchard was similar to that observed in natural environments.

Orchard experiments conducted in California and Utah during 1975-1978 examined the pollination efficacy of the blue orchard bee and factors that influenced nesting success in an orchard. Caged trees pollinated by blue orchard bees produced more fruit (almonds and apples) than uncaged trees in the same orchards pollinated by honey bees. The quality of apples from trees pollinated by blue orchard bees was higher than from trees

TABLE 1. Total nests and cells produced by *Osmia l. propinqua* released in the Hamson Orchard (1979).

Nest traps	No. available holes	No. nests	% nest utilization	No. live bees	No. dead immatures	Total cells	♂:♀ sex ratio
Laminates No. 1-200	8,372	3,852	46.0	12,138	3,839	15,977	4.2:1
No. 201-400	8,560	2,811	32.8	8,789	2,684	11,473	4.4:1
No. 401-600	8,585	4,434	51.6	14,271	4,135	18,406	4.3:1
Milk cartons	535	176	32.9	623	194	817	4.5:1
Laminates No. I-XV	595	341	57.3	1,211	337	1,548	4.1:1
3 cardboard boxes	2,362	190	8.0	546	166	712	5.4:1
Total	29,009	11,804	40.7	37,578	11,355	48,933	4.3:1

TABLE 2. Means ($\bar{x}$), standard deviations (SD) and results of ANOVA of seed number per fruit by apple cultivar and year. Sample size is 50 fruits for each cultivar, each year.

	1978		1979		1980			
Cultivar	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	*F*-value	*P*
Red Delicious	4.1a[1]	1.4	7.7b	1.4	7.6b	1.2	114.8	<0.0001
Golden Delicious	8.9a	0.8	9.4b	0.7	9.3b	0.8	6.0	<0.005
Rome	7.9a	1.1	8.7b	0.9	8.5b	1.0	8.6	<0.001
Jonathan	6.7a	1.5	8.2b	1.1	8.1b	1.2	21.3	<0.0001
McIntosh	8.3a	0.8	9.5b	0.5	9.1b	0.9	31.4	<0.0001

[1]Year means with different letters within cultivars are significantly different from each other ($P = 0.01$, LSD a posteriori test).

Trees pollinated by blue orchard bees produced more fruit and better quality fruit than trees pollinated by honey bees.

pollinated by honey bees.

Blue orchard bees were released into a number of almond and apple orchards each year during 1975-1978. Wood was the most attractive nest material tested. Dispersal rate from the orchard exceeded 50 percent when bees were released en masse versus 15 percent when bees emerged from natal nests. Dispersal rates decreased and nesting success increased when nest materials were distributed throughout orchards instead of congregating all nesting holes in a few centrally located shelters.

TABLE 3. Results of contingency table analysis of number of fruits with at least one seed in each carpel vs. fruits with at least one seedless carpel by apple cultivar across years.

Cultivar	Year	Fruits with seeds in all carpels	Fruits with one or more seedless carpels	$\Sigma\chi^2$	Prob.[1]	Partition χ^2	Prob.[1]
Jonathan	1978a[2]	26	24				
	1979a	34	16				
	1980a	33	17				
				3.23	>0.10	—	—
Red Delicious	1978a	13	37				
	1979b	31	19				
	1980b	28	22				
				14.9	<0.001	14.5	<0.001
Golden Delicious	1978a	39	11				
	1979b	46	4				
	1980b	45	5				
				4.93	0.09	4.84	<0.05
McIntosh	1978a	38	12				
	1979b	50	0				
	1980b	47	3				
				17.3	<0.001	16.3	<0.001
Rome	1978a	30	20				
	1979b	42	8				
	1980b	42	8				
				10.5	≈0.005	10.5	<0.005

[1]Prob. = Probability.
[2]Years with different letters within cultivars are significantly different from each other.

TABLE 4. Bushels of pear and apple cultivars harvested in the Hamson Orchard over a 4-year period. Honey bees were the dominant pollinator species in 1977 and *1978; Osmia lignaria* was the dominant pollinator species in 1979 and 1980.

Year	Pears (2 cultivars)	McIntosh	Red Delicious	Golden Delicious	Jonathan	Rome	Total
1977 + 1978	104	4,380	986	204	430	184	6,336
1979 + 1980	369	5,186	3,248	288	417	307	9,715

Pollination Effectiveness

Prior to 1979, the pollination effectiveness of blue orchard bees could not be studied in apple orchards because these bees competed with honey bees. It was possible to conduct this study when an apple grower in Utah agreed to replace honey bees with blue orchard bees in his isolated orchard.

We sampled fruit yield per variety and number of seeds per variety during 1978 to determine pollination by honey bees. We then compared pollination effectiveness of honey bees (1977-1978) and blue orchard bees (1979-1980) in the same orchard. The number of blue orchard bees per acre required to fully pollinate apples and the potential increase in population of these bees were also determined.

As shown in Tables 1-5, there were significant differences in the pollination efficiency of blue orchard bees and honey bees in commercial apple orchards. When a total of 6,143 female blue orchard bees (945 females per acre) was released in one apple orchard in 1979, the population of live progeny increased by 72 percent (Table 1). Pollination efficiency increased dramatically in 1979 as indicated by the increase in numbers of seeds per fruit (Table 2), seeded carpels per fruit per variety (Table 3), and fruit yields (Table 4). Although fewer bees were released into the orchard in 1980 (253 females per acre), the number of live progeny increased by 333 percent (Table 5). As shown in Tables 2-4 pollination efficiency and fruit yields were equal in 1979 and 1980 when blue orchard bees pollinated the crop (1979-1980), and were significantly higher than in 1978 when honey bees were pollinators (Table 3).

More Efficient Pollinators

Since 1980 we have attempted to develop efficient management programs for commercial populations of blue orchard bees. A number of characteristics explain why blue orchard bees pollinate apple trees more efficiently than honey bees. Most orchard crops are cross-pollinated when pollen on the underside of a bee's body is transferred to flowers. The underside of the blue orchard bee is much hairier than the honey bee, thus enabling blue orchard bees to carry and transfer more pollen grains. The blue orchard bee collects dry pollen directly from the flower to its ventral surfaces and abdominal

TABLE 5. Nests and cells of *Osmia l. propinqua* constructed in pine laminate nest blocks: Hamson Orchard (1980).

No. nest block	No. holes	No. nests	♂♂	♀♀	Dead immatures	Total cells constructed	% nest utilization	% mortality	♂:♀ sex ratio	Cells/ nest
No. 1-50 (emerged holes)	247	90	206	74	86	366	36.4	23.5	2.78:1	4.07
No. 1-50 (fresh holes)	2,100	1,280	3,573	1,786	601	5,960	60.9	10.1	2.00:1	4.66
No. 51-325 (fresh holes)	13,200	4,827	14,404	7,079	2,303	23,786	36.6	9.6	2.03:1	4.93
Total	15,547	6,197	18,183	8,939	2,990	30,112	39.9	9.9	2.03:1	4.86

scopa, whereas the honey bee usually transfers pollen to its hind legs, where it is wetted. The grooming activities of honey bees reduce the number of pollen grains available for transfer to flowers from the ventral surface of their bodies. The wetting of pollen by honey bees also reduces the amount of pollen that is transferred and decreases the viability of pollen grains.

Blue orchard bees begin daily flights at lower temperatures (2-6°F.) than honey bees, and continue foraging activities throughout the day, even during cloudy, windy, or showery conditions when honey bees cease their flights. Blue orchard bees have a strong orientation to their nest site but not to foraging plants. This means that blue orchard bees forage cultivars across rows in different areas of the orchard on each pollen-nectar foraging trip, thus visiting trees from the different cultivars in the orchard.

Blue orchard bees always land on the sexual column of apple flowers when they collect pollen-nectar, which increases the likelihood of cross-pollination. In contrast, honey bees often land on flower petals to obtain nectar from flowers. The floral anatomy of 'Red Delicious' cultivars accomodates the nectar-robbing (but non-pollinating) behavior of honey bees. The greatest increase in fruit production due to pollination by blue orchard bees occurred with the cultivar 'Red Delicious.'

The blue orchard bee is a viable, alternative pollinator of orchard crops. A successful management program for the species has also been developed. Large reservoir populations of blue orchard bees have been identified during a 15-year field-trapping program in the Intermountain region. The blue orchard bee should become an alternative, commercial pollinator species of orchard crops in Utah and the United States.

Blue orchard bees have several characteristics that make them better pollinators of apple trees than honey bees.

ABOUT THE AUTHOR

Phil Torchio is a research scientist stationed at the USDA-ARS Bee Biology and Systematics Laboratory, Logan. He has worked on the development of pollinator species for use in alfalfa seed, orchard, and blueberry crops.

BEATING THE ODDS—IDENTICAL TWINS FOR CATTLE RESEARCH

C. W. ARAVE, T. D. BUNCH, C. H. MICKELSEN and D. PURCELL

Only about 3 percent of Holstein calves are twins. Of those, about 10 percent are monozygotic (MZ) or identical twins that result when single embryos divide. MZ twins are valuable in research since they have identical genes; any differences in performance would largely be due to environmental factors. In a study comparing the effects of two treatments on a characteristic such as milk production, one pair of MZ twins may provide as much information as 22 pairs of non-related cows.

It has been difficult and expensive to obtain enough MZ pairs for a research project. We recently tried a method of splitting embryos that greatly improves the odds of obtaining MZ twins. MZ twins were used in a project studying how environmental effects during rearing affected subsequent milk production. The method involved superovulating donor cows with follicle stimulating hormone. Donor cows were artificially inseminated, the fertilized embryos were collected by flushing the reproductive tract, and the embryos were split in two. Each half (demi-embryo) was then transferred into a recipient heifer or cow (Figure 1). The reproductive cycle of recipients had been synchronized with that of the donor cow with prostaglandins (Synchromate®) prior to embryo transfer.

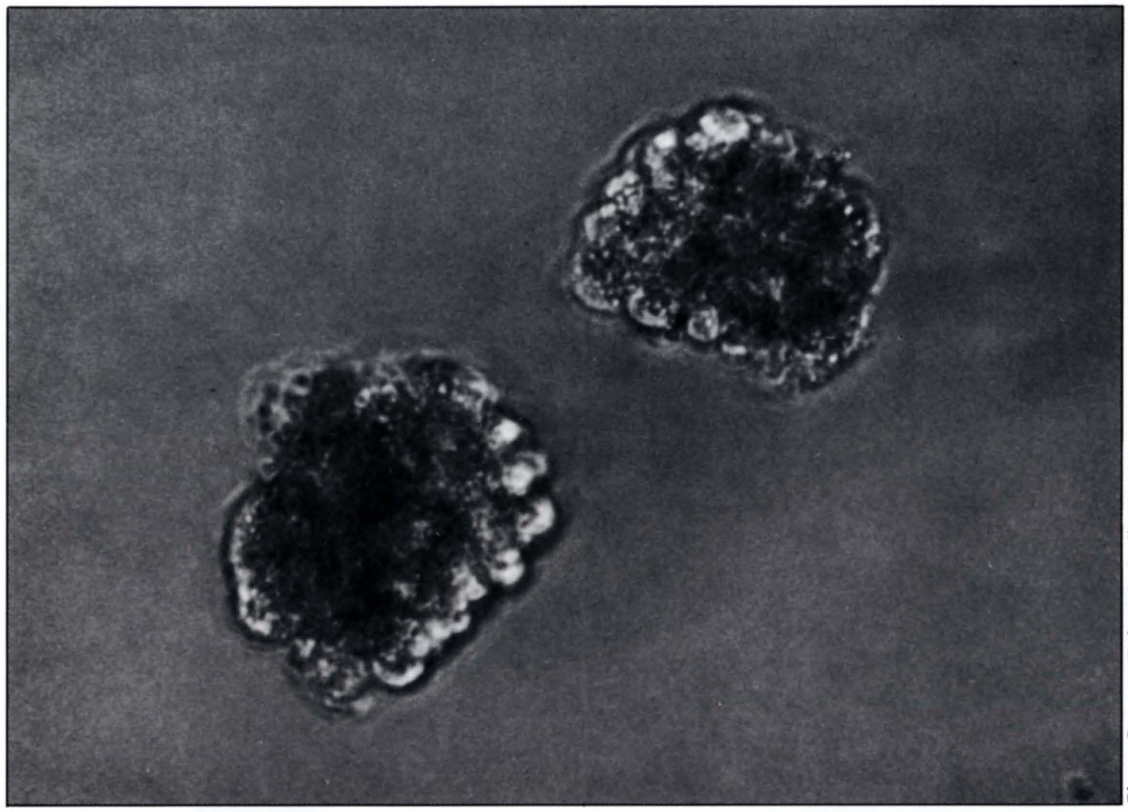


Photo: Courtesy Robert A. Godke, Louisiana State University

Figure 1. Grade 1 embryo suitable for splitting into two demi-embryos. Embryos at this age are barely visible to the human eye. Microtechniques are employed in the splitting process.

Even though the chance of obtaining MZ twins is greatly improved by using split embryos, a relatively large number of cattle are still needed to obtain an adequate number of contemporary heifers for research. In fact, the odds are 1 in 16 that a MZ heifer pair will be born and complete a first lactation. This meant that 96 embryos must be split and transferred to 192 recipients to obtain six pairs of MZ heifer twins, a reasonable number for experimental accuracy.

Since a large number of embryos and recipients could not be obtained from the University herd, we obtained help from Doug Maddox, owner of Ruann and Maddox Dairies near Fresno, Calif., a 4,000-cow operation that routinely transfers 100 embryos monthly. Em Tran, an embryo transfer (ET) company, split and transferred the embryos.

Five hundred seventy-five ova were obtained from 60 superovulated donors during 10 days of collection. Forty-three of the 60 donors provided 374 fertilized ova (embryos). Embryos represented 65 percent of total ova collected or 6.2 embryos per superovulated donor. There was a wide range (0-46 ova) in donor response to superovulation. No ova were collected from seven donors and 46 unfertilized ova were recovered from another donor. Ninety-one percent of the 374 embryos recovered were split and transferred to 181 recipients (one embryo was lost). Only embryos that received a score of grade 1 on a scale of 1 to 4 were split. The remaining embryos (283) were either transferred as whole embryos or frozen.

Pregnancy and calving percentages from split embryos were 59 and 53 percent respectively, which was slightly higher than expected. Ninety-one split embryos (181 demi-embryos) resulted in the birth of 96 calves. Sixty calves could have been expected had whole embryos been transferred. Thus splitting embryos substantially increased the utilization of genetically superior cows, especially considering that 283 whole surplus embryos were frozen or transferred.

Eight pairs of MZ twin heifers were obtained for research. In addition, one recipient gave birth to twins that were identical to a single heifer from another recipient. These triplets and a dam are shown in Figure 2. Color patterns, although not entirely genetically determined, are similar for twin pairs. Several twin calves resembled their sires (Figure 3); others resembled their dams

(Figure 2).

Environmental Effects Tested

At birth, one member of each MZ pair was placed in a polyvinyl dome hutch (Figure 4) that was spatially and visually isolated from other calves; the other twins were reared in groups of six. After 9 weeks, the calves were weaned and put together in large groups. There were no differences in average daily gain or feed intake of calves raised in different types of environments.

The learning ability of newly weaned calves was tested in a T-maze. Isolated calves outperformed group-reared calves the first 2 days of testing but there was no difference by the third day of testing. These results indicated that the environment in which calves were reared probably affected their reaction to a new situation, but this effect diminished over time.

Body measurements of heifers were taken at 15-17 months of age; there were no significant differences between rearing treatments. Heifers were also ranked according to their dominance of or by herdmates. The rankings did not differ by type of rearing environment (Table 1), another indication that the MZ twin pairs were truly identical.

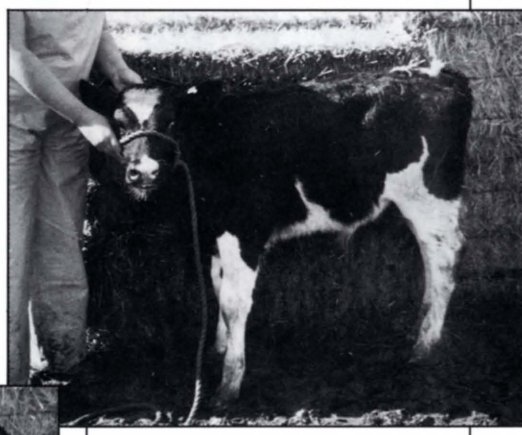
The MZ heifers were bred and are due to calve within a few months. Their milk production records will provide the final test of the similarity of MZ twins.

Another set of MZ twins has received national attention. Two bulls at American Breeders Service called "Duplicate" and "Divide" originated from a split embryo. Progeny information is now available for each of these bulls, which were born in October 1982. The first USDA Predicted Difference (the best estimate of a sire's transmitting ability) was higher for "Duplicate" (+1911) than for "Divide" (+1084). The most recent (January 1988) summary on the bulls includes more daughters and, as predicted, the PDs are converging. "Duplicate" now has a PDM of +1826 and the PDM for "Divide" is +1771. The bulls also have very similar type and performance traits. The performance of these bulls will also provide one of the most comprehensive tests of the accuracy of sire proofs.

New Cloning Techniques Developed

There is tremendous potential in the ability to freeze a demi-embryo, transfer its duplicate, obtain proof of the ability of the living animal, and, if the animal is superior, transfer its frozen duplicate.

Technological advances may make it possible to clone large numbers of embryos from a single embryo. For example, seven identical Brangus bull calves were born to recipients of a cloned embryo. Two other companies are independently working to perfect the cloning technique. Currently calves are obtained from a cloned embryo only 10-30 percent of the time, a success rate which is too low to be of commercial value. The ultimate goal is to obtain, freeze and sell cloned embryos much as frozen semen is sold today. The ability to



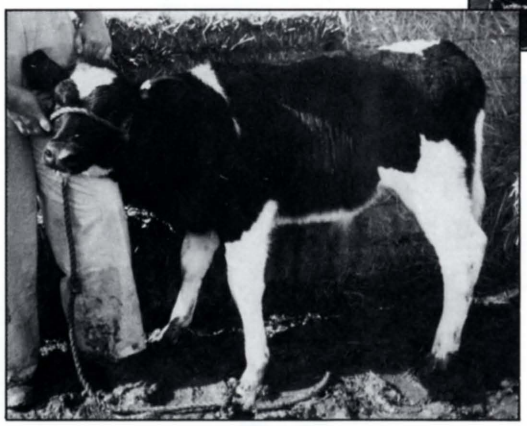


Figure 2. Top photo, dam of identical triplets; bottom two photos, full sibs to triplets showing color pattern similar to dam.

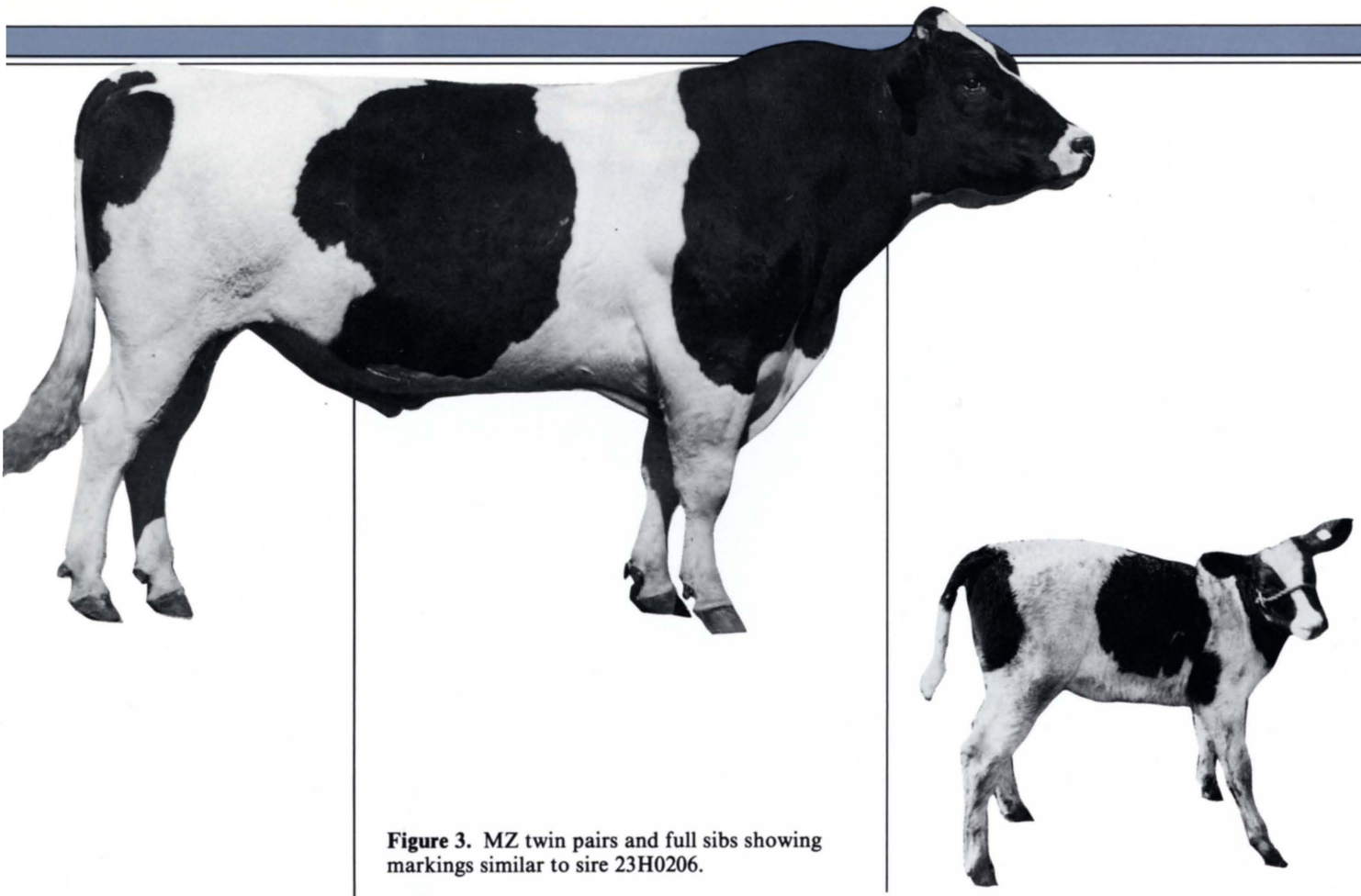


Figure 3. MZ twin pairs and full sibs showing markings similar to sire 23H0206.

TABLE 1. Dominance rank for MZ twin pairs of heifers.

	Isolated heifer no.	Rank[1]	Group heifer no.	Rank	
Pair 1	54	1	44	2	
Pair 2	52	4	93	5	
Pair 3	57	9	73	11	
Pair 4	61	12	56	10	
Pair 5	27	16	26	15	
Pair 6	030	17	25	14	
Pair 7	29	7	38	8	Triplets
Pair 8	530	6	38	8	
Pair 9	29	7	530[2]	6	
	$\bar{x}$ = 9.0			$\bar{x}$ = 9.3	

[1]A total of 17 heifers were ranked, three of which had no co-twin.
[2]Heifer no. 530 was placed in both columns for comparison of rank with co-twin.

develop animals that are genetically similar will also vastly enhance the precision of research.

ABOUT THE AUTHORS

Clive W. Arave is an associate professor in the Animal, Dairy and Veterinary Sciences Department. His research with the Agricultural Experiment Station includes genetic and management (including animal behavior) aspects of dairy cattle production.

Thomas D. Bunch is an associate professor in the Animal, Dairy and Veterinary Sciences Department. His research with the Agricultural Experiment Station has mainly involved mammalian cytogenetics and embryo research in sheep and goats.

Charles H. Mickelsen is an associate professor in the Animal, Dairy and Veterinary Sciences Department. He is a state dairy extension specialist who helps Utah dairymen solve nutritional problems in their herds.

Diane Purcell recently completed an M.S. degree in Dairy Science. Her research dealt with effects of rearing environment on growth and behavior of split-embryo calves.

The ultimate goal is to freeze and sell cloned embryos much as frozen semen is sold today.

Figure 4. Calves were placed in polyvinyl dome hutches immediately after birth and isolated by placing plywood sheets between hutches.

IRON DEFICIENCY IN PLANTS: GENOTYPE MODIFICATION OF SOIL FERTILITY

J. H. BENNETT, N. J. CHATTERTON, P. A. HARRISON and W. R. THORNLEY

Figure 1. Iron-efficient and iron-inefficient soybean plants growing in the same pot in calcareous soil. Iron chlorosis developed in the terminal leaves of the inefficient seedling after cotyledonary iron was depleted.

Physiological processes in plant roots regulate mineral uptake by chemically altering the soil close to the roots. Many of these processes are activated by environmental stress. Plant varieties that are unable to adequately carry out these essential root functions may literally starve even when minerals are abundant while more efficient varieties flourish in the same soils (Bennett et al. 1982; Olsen et al. 1981).

Plant growth is inhibited to some extent in virtually all soils due to deficiencies of one or more essential elements or, conversely, by toxic effects of high mineral ion concentrations. Imbalances can cause physiological stress and disease. Plant varieties differ in their abilities to "extract" soil nutrients and regulate mineral uptake. As a result, soil "fertility" can be determined as much by the plant varieties as by the soils in which they are grown, as is illustrated in Figure 1. Soil fertility is therefore a relative term based primarily on assessments of plant growth and productivity under specific growing conditions.

Iron Functions in Plants

Many essential chemical reactions that make energy available to living cells have evolved around processes in which iron is a key cofactor. The chemistry and metabolism of iron compounds are often studied for their evolutionary significance. As the earth's environment slowly became less anaerobic and acidic during the evolution of the earth to the more aerobic and basic conditions of today, plants were forced to develop mechanisms to solubilize unavailable iron and to regulate its uptake and utilization.

Iron becomes insoluble as the pH increases (i.e., becomes less acidic). Highly oxidizing environments can also make iron physiologically inactive. Certain plant tissues and organelles, such as chloroplasts, generate oxidants and oxyradicals that inactivate Fe in solution and precipitate unchelated Fe. Other transition-metal constituents (containing Mn, Cu, Zn, etc.) that function under these conditions may be required for metabolism and serve to protect the cells from injury. Functional iron compounds may be relegated to membrane-bound sites, vesicles, and other areas under the plant's physiological control. Table 1 lists important plant enzymes and physiochemical processes in which Fe is essential.

In neutral and alkaline soils typical of those found in Utah, the prevalent forms of iron are hydrous ferric oxides. These compounds are extremely insoluble. Although most soils contain as much as 5 percent Fe (many orders of magnitude more than is required by plants), available Fe in solution is at least a billion times too dilute for normal plant growth. Plants that remain green under these soil conditions have developed mechanisms to deal with this problem.

Obtaining Iron from Soils

Plants have evolved two general strategies to mobilize insoluble iron near roots. The mechanisms utilized by legumes and most truck crop plants (Strategy I mechanisms) differ from those of grasses and grain crops (Strategy II mechanisms). Since these Fe-solubilizing and absorption-promoting mechanisms are induced only when the plants

Plants have evolved two general strategies to mobilize insoluble iron near roots.

TABLE 1. Selected iron-containing enzymes and processes in which iron has a direct physiological role.

Physiological process	Enzyme/Function
Photosynthesis	Ferredoxin; cytochromes; chlorophyll synthesis
Respiration and general metabolism	Cytochromes, iron-sulfur proteins, iron-flavoproteins (dehydrogenases, oxidases, reductases); amino acid decarboxylase; acid phosphatase; iron-activated enzymes
Nitrogen fixation	Hemoglobin; nitrogenase
Aerobic tolerance (oxidant/oxyradical protection)	Catalase; peroxidases; superoxide dismutase
Iron storage	Phytoferritin; phosphoproteins
Other processes	Inorganic reaction Fe-phosphate precipitation, metal ion balance, inorganic redox reactions); RNA configuration

Strategy I plants respond to Fe deficiency by acidifying the soil close to the roots while Strategy II plants release natural chelators to enhance Fe uptake.

experience Fe-deficiency stress, they are called Fe stress-response functions. Green plants, particularly well-adapted weed species growing in calcareous soils, are *a priori* evidence of the existence of these vital stress-response functions.

Strategy I. Strategy I plants respond to Fe deficiency by acidifying the soil close to the roots. Unique rhizodermal cells known as transfer cells develop in the lateral roots and root hairs of Fe-stressed plants. The membrane surfaces of transfer cells become greatly enlarged and invaginated. Mitochondria increase in number and respiration rates increase.

Lateral root and root hair cells of Fe-deficient plants excrete hydrogen ions via an energy-requiring (ATPase-mediated) proton-pumping mechanism. The resulting acidification solubilizes ferric [Fe(III)] iron in the root-soil interface zone. Fe(III) is then reduced to the ferrous [Fe(II)] form that can be absorbed by the roots. When the pH is lowered, conditions are more favorable for the reduction of iron. Chemical reduction, catalyzed by reductase enzyme systems, occurs at the surfaces of rhizodermal cell membranes. The root cells of some plants also excrete chemical reductants.

Strategy II. *Graminae* species (Strategy II plants) respond to Fe deficiency by releasing nonproteinogenic amino acid Fe(III)-chelators. These natural chelators, known as phytosiderophores, enhance the uptake of Fe over a broad pH range (between 4 and 9). Our current knowledge of Strategy II mechanisms is limited, but the complexing agents appear to be preferentially produced and liberated from apical root zones. The ferrated siderophores are evidently absorbed by membrane transport carriers along the whole root system. Presumably, Fe uptake by the roots does not require the reduction of Fe(III) at the plasma membrane. Grass roots absorb iron 100 to 1000 times faster from Fe-phytosiderophores than from commonly used synthetic chelates such as FeEDTA. Plant genotypes can differ in their abilities to produce phytosiderophores and in their uptake of Fe complexes. However, Fe uptake by grasses appears to be related mainly to differences in the release of phytosiderophores into the root zone.

Non-grass species absorb iron from Fe(III) siderophores and from synthetic Fe chelates at equivalent rates. In Strategy I plants, iron uptake must be preceded by the reduction of Fe(III) to Fe(II) whether iron is supplied as Fe-phytosiderophores or as synthetic Fe-chelates.

USDA Research

Research conducted at the USDA's Forage and Range Research Laboratory in Logan, Utah, has shown how temperature affects Fe stress-response functions in warm season (respiration-intensive, Strategy I) legume plants and cool season (respiration-conservative, Strategy II) crested wheatgrass plants. Respiration rates increase with temperature, thus increasing the utilization of available substrates. Soluble sugars are the major respirable substrates translocated within plants. Our research studied influence of the carbohydrate economies of temperature-sensitive and temperature-tolerant plants on Fe stress-response functions (Bennett et al. 1988).

This review summarizes what we have learned about the responses of soybean, snap bean, and crested wheatgrass genotypes. The growth and susceptibility of soybean and snap bean plants to Fe-deficiency chlorosis can differ markedly at temperatures above 30° C. The response of overwintering crested wheatgrass plants with high sugar contents to iron stress and the induction of chlorosis in temperature-intolerant plants are also discussed.

Iron Uptake: Physiological and Environmental Interactions

Legume Physiology. Metabolic energy for root physiological functions requires the translocation of respirable substrates within the plants. Strategy I mechanisms (H^+ efflux and chemical reduction mechanisms) can be limited by the amount of photosynthates or stored reserves that are allocated to the roots. Developing seedlings depend heavily on cotyledonary reserves to become established. Seedlings are particularly at risk during the transition period when cotyledonary reserves become depleted and new leaves do not yet export photosynthates.

We tested the root acidification of germinating soybean seedlings, with and without cotyledons. The seedlings were incubated for 3 days on pH-indicating agar test plates. Only the seedlings with cotyledons were able to acidify the root media. Detaching the cotyle-

dons inhibited both root development and H^+ activity.

Soybean seedlings and other large-seeded legumes typically require several weeks of rapid growth after germination to become established. Depletion of seed reserves or premature damage to the cotyledons during this period results in poor establishment. Low reserves also limits Fe stress-response activities by the roots.

Root-shoot transport and redistribution of ^{14}C-sucrose derived from primary (exporting) leaves on a 3-week-old soybean plant grown at 25°C is illustrated in Figure 2. Two weeks after germination, radiolabeled sucrose was applied to the terminal half of the source leaf. The autoradiogram was made 1 week later. Sucrose was transported through the phloem to the roots and developing sink leaves.

Sucrose is the principal respirable carbohydrate translocated within the plant. Photosynthetic carbon-fixation reactions and major reserve carbohydrates stored in the leaves are outlined in Figure 3. Unlike legumes, cold-hardy crested wheatgrass can temporarily store large quantities of fructans (fructose-containing polysaccharides) in the vacuoles of its leaves.

The study of the uptake of radiolabeled ^{59}Fe by active roots and its redistribution within the plant showed that absorbed ^{59}Fe was transported through the conducting tissues to the growing apices and new leaves. Plant Fe did not move into or out of mature green leaves.

Iron chlorosis and Fe stress-response functions were studied in selected bush bean X pole bean genotypes known to have different responses to Fe stress at high temperatures. The chlorotic plants were F1 progeny of two promising breeding lines hybridized at the International Center for Tropical Agriculture, Cali, Columbia (Shii et al. 1980). Symptoms of iron chlorosis developed in the terminal leaves of sensitive plants when temperatures exceeded 25-30°C. At lower temperatures the hybrids remained green and developed normally. The temperature-tolerant parent genotypes did not become chlorotic under these conditions.

The snap bean hybrids were more sensitive to chlorosis because root growth was restricted and Fe stress-response activity was suppressed. Root-shoot developmental anomalies also reduced the effectiveness of iron transport and utilization. The most-sensitive seedlings had smaller cotyledons and germinated sooner. Depletion of metabolic substrates caused them to become stressed before the parent genotypes, a condition that was exacerbated at elevated temperatures. Plants grown at lower temperatures until the leaves were capable of exporting photosynthates were less susceptible to chlorosis.

Crested Wheatgrass. The reestablishment of cool-season perennial forage grasses during late winter-early spring involves different factors than those described for annual legume crop plants. Overwintering grasses store respirable carbohydrates for early spring growth in their roots and crowns. When the ground thaws in late winter, vigorous crested wheatgrass plants begin to regrow. Stored reserves supply substrates for this flush growth and for maintenance respiration during the winter. Hot (late spring and summer) temperatures cause the plants to mature and become dormant again. The new leaves of some plants develop symptoms of Fe chlorosis during warm spring weather, especially during inflorescence development and seed set.

These observations raise a number of practical questions. For example: How do winter-damaged crested wheatgrass roots supply Fe for rapid regrowth in late winter-early spring? And why do some genotypes become chlorotic during the spring while others do not?

Even though grasses are considered to be Strategy II plants, young roots of Fe-stressed crested wheatgrass can acidify their rhizospheres. However, only new adventitious roots growing from the crowns of overwintering plants, which have the highest levels of stored sugar, were active. Lawn grass seedlings treated with sucrose also acidified their rhizoshperes. Acidification was not observed in the microenvironments of older branched roots.

Fibrous root systems can be dessicated and damaged during the winter, but plant growth is nevertheless vigorous early in the spring after the soil thaws. Viable roots regenerated during this period of flush growth must support foliar growth. These roots develop rapidly from the bases of the stems in the surface layer of the soil.

New roots of grass species may be unable to support the production of phytosiderophores. Alternatively, when soils are moist during late winter and early spring, plant regrowth could benefit from root acidification

Even though grasses are considered to be Strategy II plants, young roots of Fe-stressed crested wheatgrass can acidify their rhizospheres.

Figure 2. Uptake and redistribution of radioactive ^{59}Fe by a snapbean plant. Radiolabeled areas are dark. Note that iron was distributed from the roots to the growing apices. Lateral roots were removed before autoradiography to show sites of iron absorption. Research has shown that little iron is transported into or out of older leaves.

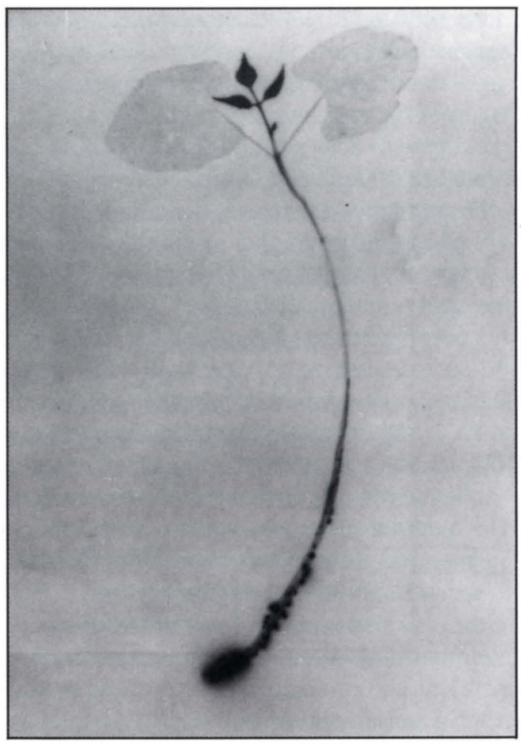


Figure 3. Diagram of carbohydrate production by a soybean plant, which affects the plant's ability to bioregulate iron uptake. Plants must have adequate carbohydrates to supply the energy required for iron uptake processes and utilization. Thus, the prevention or reversal of iron chlorosis in plants also requires other nutrients and plant hormones in addition to iron.

and reduction activities stimulated by respiratory processes. This would be particularly beneficial in the rapid reestablishment of cool-season grasses such as crested wheatgrass, whose overwintering tissues accumulate high sugar reserves (Chatterton et al. 1986).

In semiarid lands later in the year, Strategy II functions have important advantages in plants with established root systems. Strategy II mechanisms (phytosiderophore chelation and associated plant uptake) are less inhibited by high pH and high concentrations of carbonate, Ca^{++}, and Mg^{++} present in calcareous soils than are Strategy I mechanisms.

Symptoms of iron chlorosis developed in the youngest leaves of sensitive crested wheatgrass plants with spikelet inflorescences when temperatures exceeded 30°C. Chlorotic leaves were located near the reproductive sites. Lower, more mature leaves remained green. Apparently, the plant diverted plant assimilates into seed production rather than the development of new leaves. At this stage of maturity, redirection of available photosynthates to the seed heads may also reduce translocates to the roots of susceptible plants and inhibit Fe-uptake functions.

Future Applications

Interacting factors that either predispose plants to chlorosis or promote continued growth are not clearly understood. However, important progress has been made in recent years in modeling the complexities involved. New research advances and innovative computer analysis techniques make it possible to study complicated biosystems. Research concerning the relationships between environmental plant physiology and soil conditions promises to pay great dividends in agriculture, and will advance prescription farming as plant bioengineers utilize what has been learned about genotype bioregulatory systems to fit plants to existing soil conditions.

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Findings will help engineer plants better suited to existing soil conditions.

HELPING RUMINANTS GET MORE FROM LOW-QUALITY FORAGE

R. D. WIEDMEIER

Plants convert sunlight, carbon dioxide and water into two main types of carbohydrates: structural and non-structural. Non-structural carbohydrates such as starch are associated with the contents (cytoplasm) of plant cells and are totally utilized when fed to livestock. Structural carbohydrates such as cellulose and hemicellulose are associated with the cell walls of plants. They give plants their physical integrity and form.

Non-ruminant livestock species such as swine or poultry utilize very limited amounts and ruminant livestock utilize 30-90 percent of structural carbohydrates. The variation in utilization among ruminants is due to lignin, a non-carbohydrate constituent of the cell wall. Lignin is responsible for plant rigidity. The amount of lignin depends on the age and

species of plants.

Several changes account for the increase in lignin content. The lignin content of cell walls increases as plants mature, as does the amount of cell walls. There is also an increase in plant parts that are high in lignin, i.e., mature plants have a higher proportion of stem, which is relatively high in cell walls, and a smaller proportion of leaves, which contain a smaller proportion of cell walls. Lignin decreases the utilization of cell wall (structural) carbohydrates because it is a physical barrier to rumen microorganisms or inhibits the growth of these organisms.

Mature plant materials such as cereal straws and dormant winter range grasses contain large amounts of lignified cell walls and thus are poorly utilized by ruminants. These forages are very abundant. In fact, structural carbohydrates are the most abundant organic molecules on earth. It is estimated that enough protein could be produced to supply the world's needs if ruminants efficiently utilized only 5 percent of the world's supply of structural carbohydrates (Dyer et al. 1975).

By the year 2000 world population will probably increase by 50 percent (Carter 1974), which means that 50 percent more food will be required. The high crop yields during the past few decades have depended upon dwindling supplies of fossil fuels. As the need for food increases, there will be economic and social pressure to reduce the amount of edible foods fed to livestock or to feed foods only to livestock species such as swine and poultry that utilize them more efficiently. Ruminant species will depend increasingly on materials that cannot or will not be directly eaten by humans—the structural carbohydrates associated with crop aftermath, byproducts and range plants. Animal, plant and range scientists must improve ruminants' utilization of cell wall carbohydrates as non-structural carbohydrates become less available for livestock rations.

Low-quality forages usually contain little crude protein and are highly lignified. Both factors limit the proliferation of ruminal microorganisms responsible for the fermentation of structural carbohydrates (mammals do not produce enzymes for the utilization of structural carbohydrates). Ruminants fed low-quality forages must receive supplemental crude protein. Natural sources of protein are superior to non-protein nitrogen. For example, digestibility improved by 80 percent when animals were fed diets high in low-quality forage that had been supplemented with natural sources of protein (versus non-protein nitrogen) (Males 1987). The animal may not require this crude protein, but it is needed to satisfy the requirements of the rumen microorganisms (Wiedmeier et al. 1983). When rumen microbes' needs are met, they ferment structural carbohydrates to volatile fatty acids that, with the microbial cells themselves, satisfy the nutritional requirements of the animal.

However, beyond a certain point, increasing supplemental protein will not increase the utilization of structural carbohydrates (Wiedmeier et al. 1983; Pritchard and Males 1985). At that point, digestibility of structural carbohydrates is at its maximum and factors such as lignification prevent further digestion. To increase digestibility, the forages must be delignified.

Delignification

Several chemical methods have been developed to delignify low-quality forages. Most of these methods were adapted from the paper industry and involve treatment with alkali to rupture the ester bonds between lignin and structural carbohydrates. The two most studied procedures involve treatment with sodium hydroxide (Klopfenstein 1978) and anhydrous ammonia (Sundstol et al. 1978). The latter method is the most popular because it is practical and there are fewer problems with animal toxicity. These treatments usually improve animal performance, but changes in forage utilization have ranged from -52 percent (Garrett et al. 1979) to 926 percent (Lesoing et al. 1980).

A recently developed method, which involves soaking low-quality forages in an alkaline solution of hydrogen peroxide (Kerley et al. 1986), increased forage digestibility to nearly 90 percent. The procedure is costly and requires specialized equipment. Nonetheless, it demonstrated that cereal straw can be treated so it is as digestible as cereal grain.

Another method of delignification relies on microorganisms. In nature most delignification of low-quality forages and wood is the result of fungi that produce ligninase enzymes. Normal ruminal bacteria and protozoa apparently lack ligninase activity, but recently discovered ruminal anaerobic fungi may have this capability (Akin et al. 1983).

According to one estimate, the world's need for protein could be satisfied if ruminants efficiently utilized just 5 percent of the available structural carbohydrates.

Rumen microorganisms require some crude protein to form compounds that in turn satisfy the animal's nutritional requirements.

Little is known of the requirements of these fungi or how to improve their numbers in the rumen, but they may be the key in developing a more practical method to improve the utilization of low-quality forages.

Supplementation

The utilization of low-quality forages by ruminants can be improved by both treatment methods and supplementation. Supplementation is the only practical method when ruminants graze low-quality forages. As noted above, natural, pre-formed proteins are superior to non-protein nitrogen. Natural sources are superior because they are broken down in the rumen to form amino acids, some of which are branched-chain amino acids (BCAA), which are subsequently deaminated to form branched-chain volatile fatty acids (BCVFA). Ruminal bacteria that utilize structural carbohydrates require BCVFA (Bryant 1973). The digestion of structural carbohydrates improved when either the amino acids or corresponding volatile fatty acids were provided *in vitro* (Mir et al. 1986). The ruminal requirements of these factors *in vivo* have not been elucidated.

Determining Optimal Concentration

Producers can select from a wide range of natural protein supplements. However, the ability of these proteins to supply the amino acids required for the digestion of structural carbohydrates has not been determined. The following studies were designed to determine the optimum ruminal concentration of BCVFA for the digestion of structural carbohydrates. The studies involved feeding chemically treated and untreated low-quality forages to beef cows.

Release of Branched-Chain Amino Acids

Trial 1. Different protein sources are broken down (degraded) in the rumen at different rates. A portion of the protein is broken down in the rumen and the remainder passes to the true stomach or abomasum as by-pass protein. As proteins are degraded in the rumen, amino acids, carbon skeletons and ammonia are released. The rumen microorganisms then absorb these constituents and form them into microbial protein.

Most rumen microorganisms do not require specific carbon skeletons to form microbial proteins, but microorganisms that utilize structural carbohydrates require branched-chain carbon skeletons, which are released when branched-chain amino acids are degraded in the rumen. The ability of different protein sources to release these skeletons into the rumen varies with the amount of protein degraded in the rumen and the amount of branched-chain amino acids they contain.

The nylon bag or *in situ* method was employed to rate the ability of several commonly-used protein sources to release these factors. Nylon bags (10 cm × 10 cm) containing 10 g of the protein sample were placed in the rumen of cows equipped with ruminal cannulae. The bags were attached to the ruminal cannulae caps with 80 cm nylon cords, which allowed free movement in the digesta and facilitated retrieval. The pores (50 micron diameter) in the nylon bags did not impede the movement of microorganisms and ruminal fluid.

The cows were fed a diet containing 20 percent alfalfa hay and 80 percent barley straw; this resulted in a ruminal environment and microbial profiles conducive to the utilization of structural carbohydrates. Fourteen nylon bags were prepared for each of the protein sources tested and two bags were withdrawn from the rumen at 0, 2, 4, 6, 9, 15 and 24 hours after placement. The bags were then washed, dried and the contents analyzed for dry matter, crude protein and individual amino acids. The protein sources tested were corn gluten meal, soybean meal, cottonseed meal, distillers dried grain, sunflower meal and yeast autolysate. Barley and corn grain were also tested.

This test indicated how rapidly different proteins are degraded in the rumen. However, in order to more accurately determine total degradability, it is also important to know how much protein will normally be "washed-out" of the rumen, undegraded, to the lower intestinal tract. The amount of protein washed-out of the rumen depends on the density and particle size of the protein source and the ruminal digesta outflow rates.

To obtain this information, 250 g of each protein source was baked in a solution of sodium dichromate for 24 hours. The residue was washed thoroughly and dried. This dry residue was totally undegradable by rumen microorganisms and contained 3 percent chromium.

The residues were fed to the ruminally cannulated cows and rumen digesta samples were taken at 0, 1, 2, 4, 6, 9, 12, 15, 18, 21, 24, 36 and 48 hours after feeding. The solid portion of the ruminal digesta sample was separated by centrifugation, dried and analyzed for chromium content. The rate at which the protein sources were washed out of rumen could be determined by the chromium content of the digesta over time. Data from the nylon bags and chromium-marked particles along with the simultaneous equations of Orskov and McDonald (1978) were used to predict how much BCAA were released into the rumen by the different protein sources.

Trial 2. The data obtained in Trial 1 made it possible to formulate protein supplements that would release a predictable amount of BCAA into the rumen (Table 3). The four supplements that were formulated contained either 3, 4, 5, or 6 percent of these branched-chain amino acids. The composition and analysis of the supplements are presented in Table 1. The supplements were formulated to contain the same amount of crude protein and metabolizable energy and approximately the same mineral content; they differed only in their branched-chain amino acid content.

Forty dry, pregnant beef cows were placed in eight pens, five cows per pen, such that the average age, weight and stage of pregnancy of the cows in each pen were similar. Two pens were then randomly selected to receive a specific protein supplement. Cows received 3 lb. per day of the protein supplement plus 18 lb. of barley straw. One-half of the cows receiving each protein supplement were also fed barley straw that had been treated with anhydrous ammonia (Sundstol et al. 1978).

TABLE 1. Ruminal release of branched-chain amino acids from protein supplements

	Ruminal degradabilities (%)				
Item	**Total crude protein**	**Proline**	**Valine**	**Isoleucine**	**Leucine**
Corn gluten meal	44.96[a]	43.53[a]	47.25[a]	42.46[a]	36.31[a]
Soybean meal	64.63[b]	62.15[b]	63.48[b]	62.42[b]	61.78[b]
Cottonseed meal	75.02[c]	72.13[c]	72.36[c]	70.7 5[c]	69.35[c]
Distillers dried grains (sorghum)	60.24[d]	56.05[d]	57.71[d]	55.20[d]	58.53[d]
Distillers dried grains (corn)	45.26[a]	42.73[a]	48.28[a]	41.92[a]	35.47[a]
Sunflower meal	83.36[e]	83.12[e]	82.46[e]	82.77[e]	81.22[e]
Yeast autolysate	100.00[f]	100.00[f]	100.00[f]	100.00[f]	100.00[f]
Corn grain	46.37[a]	44.72[a]	49.26[a]	44.19[a]	38.71[a]
Barley grain	81.85[g]	83.04[g]	81.61[g]	89.64[g]	82.53[g]

[a,b,c,d,e,f,g]Means in the same row and column with different superscripts differ significantly ($P < 0.05$).

TABLE 2. Ruminal protein degradabilities and release of branched-chain amino acids by protein supplements.

Item	**Crude protein (%)**	**Degradable crude protein (%)**	**Branched-chain amino acids released in the rumen (%)**
Corn gluten meal	52.75	44.96	5.75
Soybean meal	47.25	64.63	5.52
Cottonseed meal	40.25	75.02	3.86
Distillers dried grains (sorghum)	28.10	60.24	3.35
Distillers dried grains (corn)	30.22	45.26	3.35
Sunflower meal	37.72	83.36	4.45
Yeast autolysate	49.89	100.00	8.70
Corn grain	7.71	46.37	0.56
Barley grain	12.37	81.85	2.40

Average daily gain increased by 21 percent when the BCVFAA content of the supplement increased from 3 percent to 4 percent.

Cows were fasted for 24 hours and weighed prior to the study, a procedure followed 90 days later at the end of the study. Blood samples taken from each cow at the beginning and end of the study were analyzed for lymphocyte proliferation, an indication of immunocompetancy. After cows were on the protein treatments for 45 days, five fresh fecal samples were collected from each pen both morning and evening for 5 days. Feed samples from each pen were also collected throughout the same period. Collected fecal and feed samples were analyzed for dry matter and acid insoluble ash. Acid-insoluble ash, the indigestible mineral portion of feeds, is a marker for nutrient digestibility (Van Keulen and Young 1977).

Considerable Variation in Degradation

Trial 1. The results of the nylon bag study presented in Tables 1 and 2 show that there was considerable variation in the degradation of the protein sources. Little of the corn-type proteins were degraded. Sunflower meal and barley proteins were extensively degraded while yeast proteins were totally degraded. Other sources had intermediate degradabilities. The degradabilities of BCAA and total protein were similar.

These results were different from studies involving cattle fed diets high in non-structural carbohydrates (Chalupa 1976; Stern et al. 1983); ruminal amino acid degradabilities of single protein sources in these studies varied from 60 to 22 percent. The differences in degradability were probably due to the proteolytic bacterial profiles associated with the diets. The findings of this study are in agreement with those of Varvikko (1986) who used grass silage and hay diets. This is the first such study involving straw diets.

Trial 2. As shown in Table 4, average daily gain increased over 3-fold when straw treated with anhydrous ammonia was fed instead of untreated straw, regardless of protein supplement. Average daily gain of cows fed the supplement containing 4 percent ruminally released branched-chain volatile fatty acid-producing amino acids (BCVFAA) was 21 percent higher than when cows were fed the supplement containing 3 percent BCVFAA.

TABLE 3. Composition and nutrient content of protein supplements, dry matter basis.

	Amino acid release			
Item	**3%**	**4%**	**5%**	**6%**
Distiller dried grains, %	36.67	13.88	—	—
Corn gluten meal, %	34.95	—	—	—
Soybean meal, %	—	58.64	20.05	0.30
Sunflower meal, %	—	—	53.16	59.63
Yeast autolysate, %	—	—	—	13.91
Corn grain, %	18.25	12.03	6.13	—
Barley grain, %	—	6.01	12.25	18.55
Monosodium phosphate, %	3.72	3.41	2.46	1.65
Calcium carbonate, %	4.12	3.77	3.66	3.64
Trace mineralized salt[a], %	1.55	1.53	1.56	1.58
Vitamin mix[b], %	0.73	0.72	0.74	0.74
Crude protein, %	34.00	34.00	34.00	34.00
Metabolizable energy, Mcal/lb.	1.29	1.25	1.25	1.25
Calcium, %	1.84	1.84	1.84	1.84
Phosphorus, %	1.40	1.40	1.40	1.40
Magnesium, %	0.21	0.31	0.59	0.63
Potassium, %	1.30	1.37	1.11	1.05
Sulfur, %	0.39	0.35	0.25	0.27
Zinc, ppm	85.62	101.04	99.73	96.98
Manganese, ppm	63.71	74.82	81.21	79.05
Copper, ppm	37.24	31.66	22.98	23.63

[a]Zinc, 3600 ppm; manganese, 3000 ppm; copper, 330 ppm; cobalt, 30 ppm, selenium, 20 ppm; salt, 96%

[b]Vitamin A, 1,000,000 i.u./lb.; vitamin D, 100,000 i.u./lb.; vitamin E, 800 i.u./lb.

There was no additional increase in average daily gain when cows were fed supplements containing 5 or 6 percent BCVFAA.

The results indicate that cattle fed diets containing large amounts of untreated straw should also receive supplemental protein that provides at least 4 percent ruminally released BCVFAA. When cows were fed treated straw, average daily gain increased by 25.6 percent when the ruminally released BCVFAA content of supplements was 5 or 6 percent instead of 3 or 4 percent. Thus, delignification, which increased the digestibility of structural carbohydrates, also increased the BCVFAA required for maximum utilization. As expected, dry matter digestibilities followed the same pattern as cow performance.

The data concerning lymphocyte proliferation are difficult to interpret. The decrease in proliferation when supplements contained 3, 4 or 5 percent BCVFAA would normally occur in cows approaching parturition. The significant increase in proliferation in cows receiving the supplement containing 6 percent BCVFAA was unexpected. Those cows appeared to be in no better condition than cows receiving the supplement containing 5 percent BCVFAA.

This difference may be due to the yeast autolysate content of the supplement. Yeast supplements have decreased shipping fever complex in feedlot calves (Ruehlow 1988) and undefined, positive nutritional benefits have also been attributed to yeast. Perhaps one of these factors in the supplement containing 6 percent BCVFAA stimulated the immune system.

Treatments to delignify structural carbohydrates can result in a more than 3-fold increase in the utilization of ruminally released BCVFAA.

TABLE 4. Performance of dry, pregnant beef cows fed diets containing treated and untreated barley straw supplemented with different levels of branched-chain amino acids.

	Amino acids released			
Item	**3%**	**4%**	**5%**	**6%**
Average daily gain, untreated straw, lb/day	0.19[a]	0.23[b]	0.32[b]	0.40[b]
Average daily gain, treated straw, lb/day	0.85[c]	1.17[c]	1.47[d]	1.51[d]
Dry matter digestibility, untreated straw, %	46.31[e]	48.92[f]	49.63[f]	49.87[f]
Dry matter digestibility, treated straw, %	58.03[g]	58.72[g]	61.84[g]	62.06[g]
Lymphocyte proliferation, untreated straw[i], × 10^3	-12.3[j]	-11.2[j]	-26.1[j]	+31.6[k]
Lymphocyte proliferation, treated straw[i], × 10^3	-10.8[j]	-12.2[j]	-9.9[j]	+14.6[k]

[a,b,c,d]Means in the same row and column with different superscripts differ significantly ($P < 0.05$).
[e,f,g,h]Means in the same row and column with different superscripts differ significantly ($P < 0.05$).
[i]Difference in the ability of lymphocytes to proliferate *in vitro* from initial sampling to final sampling.
[j,k]Means in the same row and column with different superscripts differ significantly ($P < 0.05$).

Conclusions

Ruminant livestock species are able to produce large amounts of high-quality protein foods from material unsuitable for human consumption, i.e., structural carbohydrates. When dry, pregnant beef cows are fed diets containing large amounts of structural carbohydrates, protein supplements should be formulated to meet the crude protein requirements of rumen microorganisms, and should supply at least 4 percent ruminally released BCVFAA. Treatments to delignify these feeds can increase utilization of ruminally released BCVFAA by more than 3-fold. The cost of treatment must be weighed against benefits associated with the increase in utilization. When delignification is used to increase the potential digestibility of structural carbohydrates, protein supplements should be formulated to provide at least 5 percent ruminally released BCVFAA.

ABOUT THE AUTHOR

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