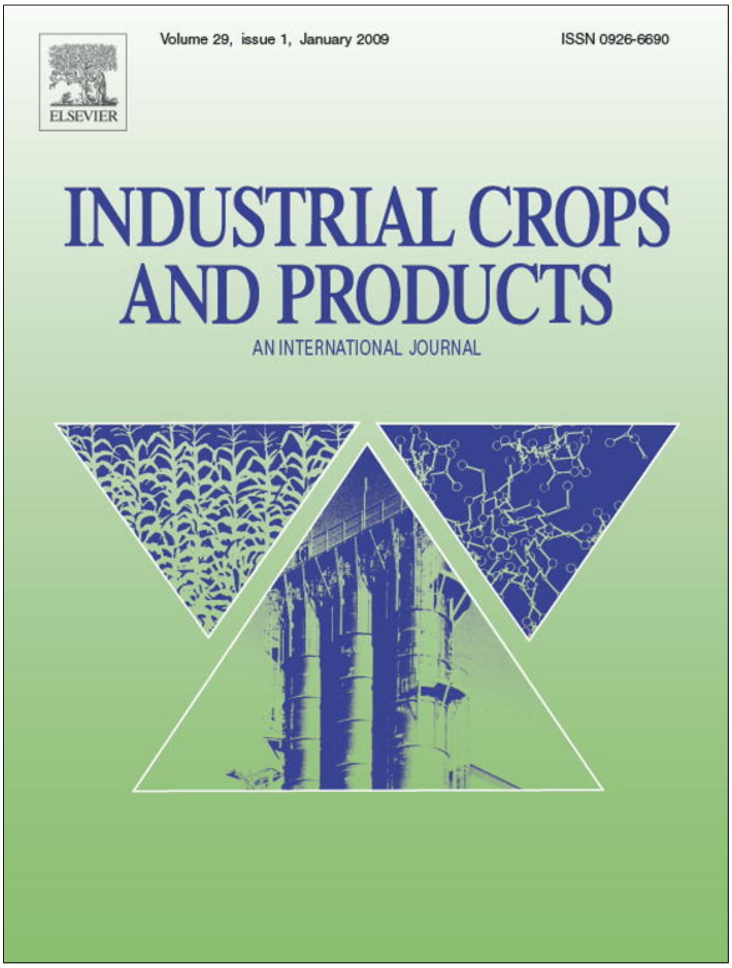
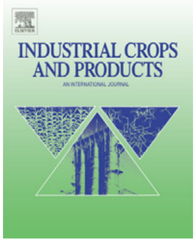

Volume 29, issue 1, January 2009
ISSN 0926-6690
ELSEVIER
INDUSTRIAL CROPS AND PRODUCTS
AN INTERNATIONAL JOURNAL

INDUSTRIAL CROPS AND PRODUCTS 29 (2009) 255–260

available at www.sciencedirect.com

journal homepage: www.elsevier.com/locate/indcrop



Plant population, planting date, and germplasm effects on guayule latex, rubber, and resin yields

T.A. Coffelt[a],*, F.S. Nakayama[a], D.T. Ray[b], K. Cornish[c], C.M. McMahan[d], C.F. Williams[a]

[a] USDA, ARS, U.S. Arid-Land Agricultural Research Center, Maricopa, AZ 85238, USA
[b] Plant Sciences Department, The University of Arizona, Tucson, AZ 85721, USA
[c] Yulex Corporation, Maricopa, AZ 85238, USA
[d] USDA, ARS, Western Regional Research Center, Albany, CA 94710, USA

ARTICLE INFO

Article history:
Received 20 February 2008
Received in revised form
16 May 2008
Accepted 23 May 2008

Keywords:
Plant population
Planting date
Germplasm
Latex
Rubber
Resin
Yield
Parthenium argentatum
Guayule
Agronomic practices

ABSTRACT

Guayule (*Parthenium argentatum* Gray) is a perennial shrub native to the Chihuahuan Desert. While guayule traditionally has been cultivated for rubber, more recently it is being cultivated for its hypoallergenic latex. Other uses including termite resistant wood products and an energy source have also been identified. However, the effects of various agronomic practices, such as planting and harvesting dates, plant spacing, cutting height and frequency, irrigation frequency, and herbicide application, on latex concentration and yield of newly developed germplasm have not been reported. The objectives of this study were to determine the yield and concentration of latex, rubber, and resin of four guayule lines planted at two populations and two planting dates. Four guayule lines (AZ-1, AZ-3, AZ-5, and 11591) were transplanted at two dates (28 November 2000 and 7 June 2001) and two plant populations (27,000 and 54,000 plants ha^{-1}). Treatments were replicated four times. Each treatment plot was subdivided into six subplots for harvesting at 6-month intervals beginning 1 year after transplanting. Results showed that transplanting date did not affect plant size or latex concentration or yield consistently. Instead, it appeared that the time of harvest (fall vs. spring) was more important. The sixth (last harvest) in the fall planting date and the fifth harvest date in the spring planting date were the optimum for plant biomass and latex, rubber, and resin concentrations and yields. The lines AZ-1 and AZ-3 were larger, whereas AZ-5 had higher latex and rubber concentrations than the control, 11591. The greater plant population (54,000 plants ha^{-1}) had higher biomass, rubber, and resin yields than the lower population (27,000 plants ha^{-1}) at the early harvest dates, but not at the later harvest dates (5 and 6). More studies must to be conducted to determine the optimum plant population and transplanting date for other newly developed guayule germplasm lines.

Published by Elsevier B.V.

1. Introduction

The genus *Parthenium*, a member of the family Asteraceae, is native to most of North America (Foster and Coffelt, 2005; Ray et al., 2005). The most promising species within *Parthenium* for commercialization as a natural rubber crop is *Parthenium argentatum* Gray or guayule, because it is the only species of *Parthenium* known to produce significant quantities of

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* *Corresponding author*. Tel.: +1 520 3166359; fax: +1 520 3166330.
E-mail address: terry.coffelt@uswcl.ars.ag.gov (T.A. Coffelt).

0926-6690/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.indcrop.2008.05.010

rubber. Guayule is a perennial shrub native to the Chihuahuan Desert of northern Mexico and southern Texas. Guayule and *Hevea brasiliensis* (A. Juss.) Muell.-Arg. are the only plants that have been grown commercially for natural rubber. Guayule's use as a natural rubber source dates back before 1500 A.D. when Native Americans used its latex to make balls for games. Guayule has been evaluated in the U.S. as a potential commercial rubber crop during at least three periods prior to the current efforts in response to various international crises (Foster and Coffelt, 2005; Ray et al., 2005).

The latest period of commercialization began in the 1980s and overlaps with the end of the preceding period. This current period was initiated when the AIDS epidemic began. In response to increased demand for latex medical products and as a result of short cuts in manufacturing processes to meet this demand, many people developed latex allergies ranging from contact dermatitis to anaphylactic shock (Ownby et al., 1994). Research showed that guayule latex did not contain the allergy causing proteins present in *Hevea* latex (Siler et al., 1996). Thus, an important difference between the current period and previous attempts at guayule commercialization is the primary product is latex rather than solid rubber. This is important, because the latex of guayule does not compete directly with less expensive *Hevea* rubber for such uses as tires, but rather with more expensive synthetic latex and *Hevea* latex in the medical devices market. Guayule latex as a natural latex has many advantages over the more expensive synthetic sources and can be made into high value medical products such as catheters, tubing, condoms, surgical balloons, and gloves.

Research has also been expanded to evaluate other potential products from guayule (Nakayama et al., 2003a,b). After latex extraction, over 90% of the plant biomass remains and must provide an economic return for guayule to be successful commercially. One area of research has focused on the use of the residual biomass to produce composite wood products. These products have been shown to be termite and wood rot resistant due to the resin in guayule (Nakayama et al., 2003a). Guayule resin has also been used to impregnate termite susceptible wood products to provide resistance to termites. These findings give guayule another use that may prove to be just as valuable as the hypoallergenic latex.

Another area that has recently received renewed attention is the use of guayule as an energy crop. Preliminary studies by Nakayama et al. (2003b) indicate that the biomass of the whole plant has higher energy values (21.77 MJ kg^{-1}) than other plant biomass sources such as corn (*Zea mays* L.) stalks, wheat (*Triticum aestivum* L.) straw, kenaf (*Hibiscus cannabinus* L.), and switchgrass (*Panicum virgatum* L.) (18.61 MJ kg^{-1}). Guayule resin (37.90 MJ kg^{-1}) has energy values comparable to the oil extracted from most oilseed crops and can be used as fuel. The entire plant biomass with both the rubber (latex) and resin removed is still equal to or higher (20.49 MJ kg^{-1}) than other plant biomass sources in energy value (Nakayama et al., 2003b).

Ray et al. (2005) recently reviewed previous work on breeding guayule, and Foster and Coffelt (2005) on agronomic research on guayule. Major advances have been made since 1970 in the development of improved guayule germplasm. Previously, the time to harvest was generally considered to be from 3 to 5 years, but with the latest germplasm releases the harvest period has been reduced to 2–3 years (Ray et al., 1999). In addition to faster growth, new lines also have higher rubber and/or resin yields than the older lines. Experimental biomass yields of 22 t ha^{-1} for these newer lines have been achieved within 2 years. All of these studies have been conducted at the standard plant population of 27,000 plants ha^{-1}.

Previous studies (Tingey and Foote, 1947; Tingey, 1952; Nakayama, 1991; Milthorpe et al., 1994; Rodriguez et al., 2002) on plant spacing have looked at plant populations both higher and lower than the current standard plant population. These studies have also involved natural stands (Rodriguez et al., 2002), direct seeded stands (Tingey, 1952), and transplanted stands (Tingey and Foote, 1947; Milthorpe et al., 1994). These studies were aimed primarily to determine plant population effects on rubber and biomass yields. Conflicting results have been reported with both increased yields associated with increased population and no increases in yield associated with increased population. Many of these differences can be associated with water, fertility, and other environmental factors. More recently, Bedane (2007) determined plant population effects on seed quality of some of the newer guayule lines. He found that plant population did affect seed yield and size, especially in younger plants (<3 years old) at low populations (<30,000 plants ha^{-1}).

The effects of various agronomic practices such as increased plant population and transplanting date on the latex content and yield of guayule have not been studied. The objectives of this study were to determine the yield and concentration of latex, rubber, and resin of four guayule lines planted at two populations and two planting dates.

2. Materials and methods

Four guayule lines were used in this study (AZ-1, AZ-3, AZ-5, and 11591). The newly released germplasm lines AZ-1, AZ-3, and AZ-5 are variable for rubber and resin concentration and biomass (Ray et al., 1999), and 11591 is an older USDA line used as the check. Seedlings were started in the greenhouse and transplanted in the field at about 3 months of age at The University of Arizona Maricopa Agricultural Center on 28 November 2000 (fall) and 7 June 2001 (spring). The initial plant populations were 27,000 and 54,000 plants ha^{-1}. The experimental design was a randomized complete block, split–split plot design with four replications. Transplanting dates were treated as locations. The whole plots were the guayule lines, the split plots were the plant populations, and the split–split plots were harvest dates. Rows were 1.0 m apart and each split–split plot was 4.3 m long for a harvested area of 4.3 m^2. One split–split plot in each split plot or harvest date was harvested every 6 months from 1 year to 3.5 years after transplanting. Two border plants were left between each harvest date to minimize any border effects.

Harvested plants were analyzed for dry biomass weight, latex concentration and yield, and rubber concentration and yield. All experiments were harvested and chipped using the method described by Coffelt and Nakayama (2007). All plants within a subplot area designated for harvest were cut within 5 cm of the soil surface and all above ground plant material chipped. Three subsamples were taken from the freshly chipped material and put on ice. One subsample was processed for latex concentration, one for rubber and resin concentration, and one for rubber quality. Latex concentration was determined by the water extraction method described by Cornish et al. (1999). Rubber concentration was determined by a modification of the organic solvent based gravimetric method of Black et al. (1983) as described by Veatch-Blohm et al. (2006). Latex, rubber, and resin yields are derived from the latex, rubber, or resin concentration multiplied by the total biomass. All values reported are based on whole plant dry weights from the harvested subplots. Data were analyzed by analyses of variance and means separated by LSD at the $P = 0.05$ level.

3. Results and discussion

Due to significant ($P < 0.05$) transplant date interactions with harvest date (plant age), line, and spacing, the results are presented separately for each transplant date (Tables 1–3). When results were averaged over all six harvest dates, four lines, and two populations, transplanting date 1 (DOT 1) was higher than transplanting date 2 (DOT 2) for plant biomass (6749 g/plot vs. 5311 g/plot, respectively), latex yield (99 g/plot vs. 80 g/plot), rubber yield (296 g/plot vs. 262 g/plot), and resin yield (592 g/plot vs. 486/plot). In contrast, DOT 2 was higher than DOT 1 in latex concentration (1.3 vs. 1.1, respectively), rubber concentration (4.6 vs. 4.0), and resin concentration (8.7 vs. 8.4). These results between dates of transplanting were larger for younger plants and tended not to be significant for later harvests or older plants (Tables 1–3).

It appeared that time of harvest (fall vs. spring) was more important than transplant date. For DOT 1 spring harvests were at 1.5, 2.5, and 3.5 years old, whereas the fall harvests were at 1.0, 2.0, and 3.0 years old. For DOT 2, the spring harvests were at 1.0, 2.0, and 3.0 years old, whereas the fall harvests were at 1.5, 2.5, and 3.5 years old. Plants of the same age, especially plants less than 2.5 years old, were larger (plant biomass) when harvested in the fall than in the spring, whereas latex concentration was higher in the spring than in the fall for the same age plants (Table 1). The other traits (latex yield, rubber concentration, rubber yield, resin concentration, and resin yield) also tended to follow the trend of higher values in spring harvests than fall harvests. These results are similar to those reported by other workers who had both spring and fall harvests (Tingey and Foote, 1947; Nakayama, 1991; Milthorpe et al., 1994).

The optimum harvest date for DOT 1, based on total latex yield, was when plants were 3.5 years old (spring 2004). This was the last harvest date for this transplanting date. Similarly for DOT 2, the optimum harvest date was harvested at the same time, but when the plants were only 3 years old. This indicates that spring harvests are probably the most desirable once the plants have reached full size. This is in agreement with other reports for rubber yields and harvest dates (Tingey and Foote, 1947; Nakayama, 1991; Milthorpe et al., 1994; Foster and Coffelt, 2005). The results also agree with previous reports (Dierig et al., 2001; Coffelt et al., 2005) that environment plays

Table 1 – Means for guayule plant biomass (grams/plot), latex concentration (%) and yield (grams/plot), rubber concentration (%) and yield (grams/plot), and resin concentration (%) and yield (grams/plot) for six harvest dates (plant age 1–3.5 years old) averaged across four lines and two plant populations within two transplant dates (DOT)

Plant age (years)	Plant biomass (g/plot)		Latex concentration (%)		Latex yield (g/plot)		Rubber concentration (%)		Rubber yield (g/plot)		Resin concentration (%)		Resin yield (g/plot)	
	DOT 1	DOT 2	DOT 1	DOT 2	DOT 1	DOT 2	DOT 1	DOT 2	DOT 1	DOT 2	DOT 1	DOT 2	DOT 1	DOT 2
1.0	2203d[1]	1602c	0.3d	0.8c	6d	14c	1.4d	4.1c	30d	67d	6.3d	7.1b	142d	115c
1.5	3104cd	3952b	0.9c	0.4d	26bcd	17c	4.6b	2.7d	136cd	104d	8.0bc	7.4b	247cd	297bc
2.0	5481bc	3269bc	0.4d	1.5b	23cd	52c	2.9c	4.8bc	153c	154cd	7.9c	9.6a	442bc	321b
2.5	4728bcd	4777b	1.5b	1.0c	67bc	47c	4.5b	4.8bc	199bc	223c	9.6a	9.4a	464bc	459b
3.0	6705b	9555a	1.2b	2.3a	76b	219a	4.8b	6.3a	304b	574a	9.5a	9.7a	638b	935a
3.5	18272a	8709a	2.4a	1.5b	398a	127b	5.6a	5.3b	952a	447b	8.8ab	9.1a	1617a	789a

[1] Means within columns followed by the same letter are not significantly different ($P = 0.05$).

Table 2 – Means for guayule plant biomass (grams/plot), latex concentration (%) and yield (grams/plot), rubber concentration (%) and yield (grams/plot), and resin concentration (%) and yield (grams/plot) for four lines averaged across six harvest dates and two plant populations within two transplant dates (DOT)

Line	Plant biomass (g/plot)		Latex concentration (%)		Latex yield (g/plot)		Rubber concentration (%)		Rubber yield (g/plot)		Resin concentration (%)		Resin yield (g/plot)	
	DOT 1	DOT 2	DOT 1	DOT 2	DOT 1	DOT 2	DOT 1	DOT 2	DOT 1	DOT 2	DOT 1	DOT 2	DOT 1	DOT 2
11591	4573b[1]	3097c	1.2ab	1.1b	78a	51a	4.3ab	5.2a	231a	219a	6.8b	6.8c	327c	274c
AZ-1	8002a	6250ab	1.1ab	1.3b	124a	92a	3.6bc	4.2b	334a	287a	8.6a	9.0b	719ab	583ab
AZ-3	9884a	6815a	0.8b	1.0b	113a	89a	3.1c	3.8b	362a	282a	9.0a	9.2ab	900a	652a
AZ-5	4536b	3055bc	1.4a	1.7a	82a	86a	4.8a	5.5a	256a	257a	9.0a	9.8a	421bc	434bc

[1] Means within columns followed by the same letter are not significantly different ($P = 0.05$).

Table 3 – Means for guayule plant biomass (grams/plot), latex concentration (%) and yield (grams/plot), rubber concentration (%) and yield (grams/plot), and resin concentration (%) and yield (grams/plot) for two plant populations averaged across six harvest dates and four lines within two transplant dates (DOT)

Plant population (plants ha^{-1})	Plant biomass (g/plot)		Latex concentration (%)		Latex yield (g/plot)		Rubber concentration (%)		Rubber yield (g/plot)		Resin concentration (%)		Resin yield (g/plot)	
	DOT 1	DOT 2	DOT 1	DOT 2	DOT 1	DOT 2	DOT 1	DOT 2	DOT 1	DOT 2	DOT 1	DOT 2	DOT 1	DOT 2
27000	6082a[1]	4616b	1.1a	1.3a	87a	71a	3.8a	4.7a	255a	225b	8.2a	8.7a	517a	424b
54000	7415a	6005a	1.1a	1.2a	112a	97a	4.1a	4.7a	336a	298a	8.5a	8.7a	667a	548a

[1] Means within columns followed by the same letter are not significantly different ($P = 0.05$).

a significant role in determining latex, rubber, and resin concentrations and yields.

The AZ-1 and AZ-3 plants were larger than the AZ-5 and 11591 plants (Table 2). The AZ-5 line was higher and the AZ-3 line lower in latex and rubber concentration, but latex and rubber yields were generally similar among lines. Because latex and rubber yields are determined both by plant biomass and latex and rubber concentration, these results show that larger plants with a lower latex or rubber concentration can yield as much latex or rubber ha^{-1} as smaller plants with higher latex or rubber concentrations. For latex and rubber extraction efficiency, it may be more desirable to utilize lines with higher concentrations. More research will need to be done to determine whether this is true. If the plant biomass is being utilized for other purposes such as for resin extraction, composite board manufacture, or as an energy source, then the higher biomass lines may be more desirable. The higher biomass lines also generally grow faster that can reduce weed competition early in the growth of plants. The AZ lines were all higher in resin concentration and yield than 11591. This is similar to previous reports for these lines (Ray et al., 1999).

Plant population (spacing) did not significantly affect the latex, rubber, or resin concentrations at either transplant date (Table 3). The higher plant population was significantly higher in plant biomass for the second transplant date, which then also resulted in significant differences between the two plant populations for rubber and resin yields for the second transplant date (Table 3). These differences were not present for the fall transplant date. However, for younger plants (<2.5 years old) the higher plant population did have higher plant biomass and correspondingly higher yields of latex, rubber, and resin for both transplants dates (data not shown). Thus, the differences between plant populations tended to decrease as the plants grew and filled in the space between plants. As guayule becomes commercialized and prices paid to growers are set, it will be possible to determine whether the increased biomass in early growth offsets the increased cost of more transplants.

These results are similar to previous studies on the effects of increased plant populations on guayule biomass (Tingey and Foote, 1947; Tingey, 1952; Nakayama, 1991; Milthorpe et al., 1994; Rodriguez et al., 2002; Foster and Coffelt, 2005). They differ from a recent report (Bedane, 2007) of studies in Australia on guayule seed quality and size where lower populations produced more seed and heavier seed. However, the results were similar in that these effects were not significant as plants aged. The highest plant population used in the study by Bedane (2007) was approximately the same as the lowest in this study, which may also account for more differences observed in younger plants in that study than in this study.

Differences in results reported for the effects of plant population on guayule biomass and rubber yields may be due to method of planting—direct seeded vs. transplant vs. native stand. Rodriguez et al. (2002) did not find any differences in yield when working with native stands without irrigation, whereas Tingey and Foote (1947) and Tingey (1952) using direct seeding and transplanting of selected lines found significant plant population effects. In contrast, Milthorpe et al. (1994) and the current study found effects only in younger plants. Work by Tingey and Foote (1947) indicated that for increased populations to result in increased rubber and biomass yields, the plants must have adequate water and fertilizer. If these inputs are limiting, this may explain the different results between studies. Plants in this study received ample irrigation and did not exhibit any nutrient deficiency symptoms in the foliage indicating that fertility levels were probably adequate for optimal growth. Apparently, the interplant competition between the older plants in our study was so large that any differences due to plant populations were masked. This was visually evident at the last harvest dates when individual plants at the low population were noticeably larger than individual plants at the high population.

The amount of latex observed in these studies was lower than that expected based on the report by Cornish et al. (1999) that 60–100% of the total rubber was latex in the plant. The difference in the range of values reported by Cornish et al. (1999) and the values reported here could be due to at least three factors. First, the method of extraction is completely different for the two forms of rubber—total (solid) vs. latex. The determination of total rubber is by a cyclohexane solvent extraction process that extracts everything that is soluble in cyclohexane from the plant. This extract is composed mostly of rubber, but other compounds are also present. The determination of total latex is by a water based extraction method. This method does not include any rubber particles that are present as solid rubber in the plant or any latex rubber converted to solid rubber during the extraction process. Extraction of more compounds than rubber in a sample by cyclohexane could result in higher values for total rubber compared to total latex. Loss of latex during the water extraction method could also result in lower total latex values compared to total rubber values. Second, the values could differ from those reported by Cornish et al. (1999) because different plant materials were used. Cornish et al. (1999) used plant branches from older plants (>3 years), whereas we used whole plants in this experiment. Because leaves are known to have little or no latex in them and can make up to 50% or more of the plant weight, we would expect a lower latex content than defoliated branches. Third, the analyses were performed in two different labs on two different subsamples. While precautions were taken to make sure both subsamples were representative of the original plant biomass, some sampling differences could have occurred. The results presented in this study are not meant to be a measure of rubber balance, but rather a comparison of the different treatments and their effects on latex and total rubber. We feel the results presented are a good measure of the relative differences among treatments for both total latex and rubber.

4. Conclusion

This is the first study to report the effects of plant population and transplanting date on latex content and yield in guayule. The results for latex concentration and yield were similar to those for rubber concentration and yield, indicating that results from previous studies analyzing only for rubber concentration and yield can probably be used to infer what effects the factors studied would have on latex concentration and yield. Because latex analysis is more difficult than rubber alone, this should make agronomic studies easier to conduct.

Results from this study showed that harvest date (spring vs. fall) had more of an effect on plant biomass, and thus, yield of latex, rubber, and resin than time of transplanting with spring harvests giving the best yields. The biomass of the new lines AZ-1 and AZ-3 was generally larger than the new line AZ-5 and the older line 11591, whereas the lines AZ-5 and 11591 were higher in latex and rubber concentrations than the lines AZ-1 and AZ-3. All the AZ lines were higher in resin concentration and yield than 11591. Plant population had little effect on latex, rubber, and resin concentration, but the higher population did produce more biomass, and thus, yields of latex, rubber and resin, especially in the spring transplant date and early harvests of both transplant dates.

Acknowledgements

The authors thank Amy Clark, Maureen Jacobs, Marcal H. A. Jorge, Greg Leake, Melinda Main, Ghislaine Majeau, Valerie Teetor, Maren Veatch-Blohm, Stephen Vinyard, Cynthia Walker, and Elisabeth Wittenberg for technical assistance. The research was supported in part by a grant from the Fund for Rural America #97-36200:FRS306180 and a grant from the Initiative for Future Agriculture and Food Systems #00-52104-9660.

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