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Kudzu [*Pueraria montana* (Lour.) Merr. Variety *lobata*]: A new source of carbohydrate for bioethanol production

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ABSTRACT

We determined the amount of standing biomass of kudzu (*Pueraria montana* var *lobata*) in naturally infested fields in Maryland and Alabama, USA. At each site, we evaluated the carbohydrate content of roots, stems, and leaves. For a third site from Georgia, we evaluated the carbohydrate content of kudzu roots of varying diameters. Belowground biomass in Alabama exceeded 13 t ha^{-1}, and contained an average of 37% fermentable carbohydrates (sucrose, glucose, and starch). Roots from Georgia of all size classes contained over 60% fermentable carbohydrates. Biomass and carbohydrate levels in roots from Maryland were low compared to plants growing in Alabama and Georgia, producing 5 t ha^{-1} of roots with 20% non-structural carbohydrate. Stems from Alabama and Maryland contained 1–3% carbohydrates. Based on the yield and carbohydrate content, we estimate wild kudzu stands in Alabama and Georgia could produce 5–10 t ha^{-1} of carbohydrate, which would rival carbohydrate production from maize and sugar cane fields. If economical harvesting and processing techniques could be developed, the kudzu infesting North America has the potential to supplement existing bioethanol feedstocks, which could be of significance to the rural economy of the southeastern USA.

1. Introduction

Kudzu (*Pueraria montana* var *lobata*) is an invasive vine from eastern Asia which has colonized much of the southeastern United States. Kudzu currently infests approximately 30,000 km^2 and is increasing its areal coverage by over 500 km^2 per year [1]. Kudzu costs the US economy over $500 million per year as lost crop and forest productivity, expenditures for control, and damage to property [2]. Environmental damage associated with kudzu is severe, such that it is considered one of the 100 worst bioinvaders in the world [3]. Kudzu forms dense stands of vines that produce a leaf area index of 4–8, with growth rates that can exceed 2 m per week [4,5]. As a result of its aggressive growth, kudzu overtops all forms of vegetation, and frequently forms a dense monoculture that destroys habitat for most native plants and animals. Along its southern tier of infestation from Louisiana to Georgia, "kudzu-scapes" exceeding a hectare in area are common. Kudzu also alters regional air quality by emitting isoprene [6], and can reduce water quality by fixing large amounts of nitrogen that leach into surrounding watersheds [1]. Because of these negative aspects, kudzu has earned a notorious reputation in the USA, where it has been referred to as "the plant that ate the South", and a "vegetal form of cancer" [7].

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The success of kudzu is due in part to its production of large underground storage roots. These are rich in starch and other carbohydrates, commonly exceed 10 cm in diameter, extend over 2 m in length, and weigh up to 180 kg [2,8]. Starch contents near 25% of root dry mass have been noted [9,10]. Stored carbohydrates in the roots support fast growth of kudzu vines in the spring following the break in dormancy, allowing for the rapid development of a dense canopy that shades competing vegetation [1,4]. The vines and roots also produce high-quality fiber for paper and textiles [11]. In Asia, the roots are harvested for a starch that is used as a thickening agent in local cuisines, and the plant is cultivated on a small scale [7]. No sustained commercial use of American-grown kudzu has been documented, although it is occasionally used as forage for livestock [11]. Numerous proposals for exploiting North American kudzu for paper and textile production, starch production and fuel sources have been published, but little has come of them [10–12]. In North America, the large stands of kudzu are left alone, except where costly control measures are implemented. These often fail, because kudzu can quickly regrow from any stem fragment or crown that escapes control efforts [2,5].

In response to the high price of petroleum and new initiatives by governments to reduce dependence on imported fossil fuels, the demand for bioethanol feedstocks in the Americas and Europe has risen dramatically in recent years. In 2006, 17% of the US corn output was diverted to ethanol production [13]. Between January 2006 and 2007, demand for bioethanol rose by over 34%, and the existing production capacity should double when facilities now under construction are brought on line in the near future [13]. With the dramatic rise in bioethanol demand, concern has arisen that food supplies are being affected, leading to significant price spikes [14]. To meet the demand, new and more efficient sources of fermentable biomass are being sought, primarily through facilitating cellulose breakdown of high-productivity C_4 crops such as maize, sorghum, *Miscanthus*, and switchgrass [15,16]. In contrast to the high emphasis on existing crops and cellulosic fermentation, there has been much less consideration given to identifying alternative sources of fermentable biomass, notably invasive species such as kudzu that have already produced large standing stocks of carbohydrate.

With the increasing demand for liquid biofuels and the widespread distribution of kudzu across eastern North America, we hypothesize that kudzu roots may be a significant new source of carbohydrate for the production of liquid biofuels. Tanner et al. also suggested using kudzu as a biofuel during the energy crises of the late 1970s [12], but to no avail. Here, we report the results of a study of the biomass and carbohydrate contents of kudzu from a location in Rockville, Maryland (near the northern edge of kudzu's distribution in North America), from Lee County, Alabama, where kudzu exhibits high productivity, and from a productive kudzu field in Statesboro, Georgia, USA.

2. Materials and method

2.1. Experimental sites

Plant material was collected from three locations. Leaves, vines, and roots from a mature (seed-bearing) kudzu population on the USDA experimental farm in Rockville, Maryland (39°02′35N, 76°54′25W), were sampled in November 2007 following leaf senescence. Roots were excavated by shovel from three 1 m^2 plots to a depth of 60 cm. All roots in the excavations were collected and weighed.

Leaves, vines (= stems), and roots of kudzu were sampled from an infested field in Lee County, Alabama (32°25′51N, 85°25′48W) during December 2006. Prior to sampling, four 2 by 5 m plots were randomly delineated within a large kudzu field. Aboveground biomass from each plot was determined by collecting all vegetative material above the ground line. The biomass was then sorted to remove non-kudzu material and to separate the kudzu leaves and vines. Fresh weight was then recorded for leaves and vines. A sub-sample of leaves and vines was collected and oven dried at 55 °C to a constant weight. Roots from each plot were harvested to a depth of 50 cm using a Bobcat front-end loader. Roots were sifted by hand from the scoop of the front loader and then brought to the lab where they were treated the same as the aboveground material.

Samples from both locations were frozen in liquid nitrogen, and stored in a −80 °C freezer to await freeze drying. Material was freeze dried for 5–9 days (depending on the material; roots took longer), then ground to 2 mm mesh using a Cyclone sample mill, and assayed for non-structural carbohydrate content.

To evaluate the carbohydrate content of different sized roots, a third collection was made from one location in a kudzu field in Statesboro, Georgia (32°26′17N, 81°46′50W) during March 2007. Roots were extracted by shovel and sorted into three categories, small (9–15 mm diameter), medium (18–30 mm diameter), and large (30–50 mm diameter). Roots were dried to a constant weight at 65 °C and ground to a powder for carbohydrate assays. To test whether carbohydrate levels varied across the roots, a 1 cm disk was extracted from the middle core of the large roots (>30 mm in diameter), and a second 1 cm disk from just under the epidermis at the root periphery. These disks were also dried and ground to a powder for carbohydrate assay.

2.2. Chemical analysis

For each sample from Alabama and Maryland, approximately 50 mg of dried tissue was extracted at room temperature with 2 ml of methanol–chloroform–water (12:5:3) using a ground glass tissue homogenizer. The homogenates were centrifuged at 5800*g* for 15 min. The pellets were rinsed with 1 ml 80% ethanol and centrifuged as described above. The supernatant fractions were combined and partitioned with 1 ml H_2O and 1 ml chloroform. The clear aqueous fraction was evaporated to dryness under a stream of N_2 at 37 °C. The dried samples were dissolved in 1 ml deionized H_2O and stored at −20 °C prior to assay. Pellets were suspended in 2 ml H_2O and gelatinized in a boiling H_2O bath for 30 min. Starch was converted to glucose at 50 °C in 1 ml reactions containing 1 mg α-amylase and 0.05 mg amyloglucosidase (A-2771 and A-7255, respectively, Sigma Chemicals, St. Louis, MO, USA) and 0.1 M sodium acetate buffer, pH 5.0. Both commercial enzyme preparations were purified by column chromatography prior to use. Sucrose, glucose and the glucose liberated from starch were quantified using coupled enzyme assays as described previously [17].

Samples from Georgia were ground in a Wiley mill with a #4 mesh and assayed for carbohydrates according to Hendrix [18]. Soluble sugars were extracted by three washes of 80% ethanol at 80 °C. The supernatant was centrifuged at 10 000*g* for 10 min with a 50:50 mix of activated charcoal and polyvinyl-poly-pyrrolidone (PVPP) to remove impurities. A small aliquot of the purified sugars was dried overnight and reconstituted with distilled water for analysis. The starch remaining in the samples was boiled in 0.2 M KOH for 1 h, followed by a 30 min digestion at 85 °C with α-amylase (A-3403, Sigma Chemicals) and a 1 h digestion at 55 °C with amyloglucosidase (A-3042, Sigma Chemicals). An aliquot of the starch supernatant was spun with the activated charcoal:PVPP mix. Glucose, fructose, sucrose, and starch concentrations were estimated spectrophotometrically at 340 nm (Hewlett Packard 8452A) by measuring the content of glucose equivalents released during extraction and enzymatic digestion (Sigma glucose kit GAHK-20, Sigma Chemicals). Phosphoglucose isomerase (Sigma Chemicals, P5381-1 KU) was used to convert fructose to glucose. Sucrose concentrations were determined by adding invertase (I-4504, Sigma) to samples after the fructose analysis.

3. Results

Carbohydrate levels varied substantially in kudzu organs. Vines had the lowest values of starch and soluble sugars, with 1.5% soluble sugars (glucose+sucrose) being observed in both the Alabama and Maryland collections (Table 1). Starch values in the vines were 1–2% in both locations. Soluble sugar content in the senescent leaf material still attached to the vines was 1.2% in Alabama and 4.4% in Maryland (Table 1). Leaf starch content was 1.8% in Alabama and 5.3% in Maryland. Total non-structural carbohydrate (TNC) in leaves ranged between 3% in Alabama to 9.7% in Maryland, while TNC values in vines from both locations were near 3% (Table 1).

The vast majority of carbohydrates were present in the roots. Soluble sugar content was 7.1% in Alabama roots and 9.0% in the Maryland collections, with over 80% of the soluble sugars occurring as sucrose (Table 1). In the roots collected from Georgia in early spring, just prior to bud-burst, the soluble sugar pool was 18–26% of the root dry matter, with most sugars occurring as glucose and sucrose. In small roots, 75% of the soluble sugar was glucose, while in the large roots, roughly half of the soluble sugar was glucose and the other half was sucrose (Fig. 1). Fructose contributed little to the sugar pool in kudzu roots from Georgia.

In Alabama, the mean starch content of the roots was near 30% (Table 1), with the range of the four plots being 9.5–39% (not shown). Starch content in the Maryland roots was relatively low, being 11% of dry weight (Table 1). In Georgia, the sampled roots had much higher starch levels, being near 46% in roots of all size classes (Fig. 1). All carbohydrates were pooled to calculate TNC; mean TNC comprised 20% of the root dry weight in Maryland, 37% of the root dry weight in Alabama, and 68% of the root dry weight at the Georgia site. There was no difference in the carbohydrate content between the core and edge regions of large roots from the Georgia site (data not shown).

Table 1 – Mean carbohydrate content of kudzu organs harvested in December 2006 in Alabama, and October 2006 in Maryland, USA

Plant part	Lee County, Alabama		Rockville, MD	
	$\mu mol\ g^{-1}$	% of dry matter	$\mu mol\ g^{-1}$	% of dry matter
Glucose				
Leaf	11±12	0.2	84±13	1.5
Vine	9±1	0.2	8±2	0.2
Root	52±12	0.9	33±7	0.6
Sucrose				
Leaf	30±10	1.0	85±12	2.9
Vine	38±9	1.3	37±9	1.3
Root	182±41	6.2	245±18	8.4
Starch				
Leaf	98±5	1.8	291±36	5.3
Vine	107±18	1.9	72±19	1.3
Root	1646±339	29.7	615±61	11.1
Total non-structural carbohydrates				
Leaf	139	3.0	460	9.7
Vine	154	3.4	117	2.8
Root	1880	36.8	893	20.1

Mean±SE. Starch and sucrose values are derived from glucose equivalents following enzymatic digestion.

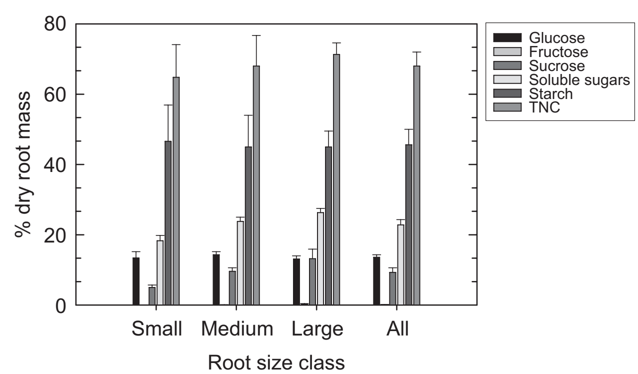

Fig. 1 – Carbohydrate content in kudzu roots of varying size classes. Roots were collected in March 2006 at Statesboro, Georgia, USA. The small size class is 9–16 mm diameter, the medium size class is 18–30 mm, and the large size class is 30–50 mm diameter. Mean±SE, *N* = 5. Differences between size classes were non-significant at $p < 0.05$ except for sucrose, where large roots had significantly more sucrose than small roots (by one-way ANOVA and a Tukey's means test).

Biomass in fields dominated by kudzu was estimated for the Alabama and Maryland sites (Table 2). Aboveground dry weight was 5.0 t ha^{-1} at the Alabama site, while belowground dry weight was 13.6 t ha^{-1} in the top 50 cm of soil. At the Alabama site where TNC was 37% of belowground dry biomass, we estimate there would have been 5.0 t ha^{-1} of

Table 2 – Biomass estimates for kudzu in Lee County, Alabama (AL) harvested in December 2006, and Rockville, Maryland (MD) in October 2006 (belowground dry weight only)

Carbohydrate	Site	Mass, ($t\,ha^{-1}$)	TNC estimate, (%)	Yield, ($t\,ha^{-1}$)
Aboveground fresh weight	AL	8.8 ± 0.9		
Aboveground dry weight	AL	5.0 ± 0.7	3	0.15
Belowground fresh weight	AL	36.1 ± 1.7		
Belowground dry weight	AL	13.6 ± 0.6	37–68	5.0–9.3
Belowground dry weight	MD	5.2	20	1.0

Estimates of total non-structural carbohydrates are compiled from ranges of mean estimates in Table 1 and Fig. 1. The 68% TNC value is derived from the Statesboro, Georgia collections. Mean ± SE for yield in the Alabama plots.

fermentable carbohydrates. If the Georgia site had similar amounts of belowground biomass, the yields would have exceeded 9 $t\,ha^{-1}$ of fermentable carbohydrate. The Maryland site had 5.2 $t\,ha^{-1}$ of root mass, which would have yielded approximately 1 $t\,ha^{-1}$ of usable carbohydrate, assuming a carbohydrate content of 20% (Table 2).

4. Discussion

Kudzu roots of all sizes are capable of producing between 20% and 68% TNC. With this range of TNC content, and the root biomass harvested from kudzu sites in Maryland and Alabama, we estimate that infested land can produce 1–9 $t\,ha^{-1}$ of fermentable carbohydrate in the form of glucose, sucrose, and starch. The low estimate of 1 $t\,ha^{-1}$ corresponds to a Maryland field near the northern range limit of kudzu where productivity is reduced by a less favorable climate. The high estimates are from sites in the core of the distribution of kudzu in North America, where high yields are promoted by long warm growing seasons. Our estimates are likely underestimates, for kudzu roots extend well below the top 50 cm of soil, and the largest roots are noted to extend over 2 m in depth [7]. While there are no known estimates of kudzu root mass below 0.5 m, our experience in kudzu patches indicates that a 20–50% higher yield could be possible, given feasible harvesting technology. If so, a carbohydrate yield of 5–12 $t\,ha^{-1}$ is a reasonable estimate for productive, well-established kudzu stands in the southern USA.

These upper yield estimates compare well with the major bioethanol crops in use now. The most widely used bioethanol crop is sugar cane, which can produce up to 80 $t\,ha^{-1}$ of dry mass with a sugar content of 10–15%. This corresponds to 8–12 $t\,ha^{-1}$ of sugar and 6.4 $m^3\,ha^{-1}$ of bioethanol, assuming a conversion efficiency of 80 $l\,t^{-1}$ of dry mass [19,20]. Sweet sorghum can produce up to 10 $t\,ha^{-1}$ of sugar, while sugar beets produce 8–10 $t\,ha^{-1}$ [19]. Maize, the most commonly used bioethanol feedstock in North America, produces 7–10 $t\,ha^{-1}$ of fermentable grain that is 65% carbohydrate. Maize commonly yields 5–7 $t\,ha^{-1}$ of fermentable carbohydrate, which converts to 2–3 $m^3\,ha^{-1}$ of bioethanol [21]. We project that the conversion efficiency for kudzu would be similar to that of cassava roots (180 $l\,t^{-1}$ of dry biomass [19]). Cassava roots are 28–35% carbohydrate [19], similar to that observed for kudzu from the Alabama site. A field of kudzu that produced 13.6 $t\,ha^{-1}$ of dry mass as we observed in Alabama would thus yield about 2.5 $m^3\,ha^{-1}$ of bioethanol, comparable to that of maize.

In the absence of efforts to cultivate kudzu, we envision it could be a useful supplement to existing bioethanol feedstocks in the USA, rather than a major replacement of those stocks. Much of the estimated 30,000 km^2 that kudzu infests in North America would not be amenable to economic harvesting, as the ground is too steep or rocky. As a rough estimate of the potential contribution of kudzu to bioethanol production in the USA, we assumed one-third of the currently infested land could be economically harvested. Assuming a mean dry matter yield of 7 $t\,ha^{-1}$ (the midpoint of the Maryland and Alabama figures), and a conversion efficiency of 180 $l\,t^{-1}$ of dry mass, the potential bioethanol production for kudzu in the USA would be $1.6 \times 10^6\,m^3$, or about 8% of the USA bioethanol production in 2006 [13].

The highest carbohydrate concentrations in kudzu roots are present during the dormant season from October to March. December has been reported to be the peak period for kudzu starch content, although this is based on limited study [11,22]. We observed high carbohydrate levels in material collected in Georgia during March, indicating harvests could continue until bud break. Root size had little effect on the starch content of roots, as we observed small roots had similar carbohydrate contents as large roots. Thus, all kudzu roots thicker than 1 cm appear to be utilizable.

A major unknown factor is whether kudzu could be economically exploited. Because of its invasive growth habit, input costs in terms of fertilizers, pesticides, planting, and stand management would be nil. Harvesting could be expensive, given the need to excavate the roots. An important consideration is whether existing farm equipment could be easily adapted to harvest and process kudzu roots, thereby avoiding large capital investments. No technique exists for processing kudzu roots to biofuels, so it is difficult to speculate on these costs. Kudzu starch is easy to extract, but the roots are rich in tannins which could inhibit microbial activity, although these are easily leached [11,12]. The 25–32% residual fiber content in the roots could be valuable for paper and textile manufacturing or cellulosic ethanol production [10–12]. Kudzu fiber is currently used in Asia for paper and textiles [7], and hence it could become an export commodity. The stems are also a rich fiber source, or could be burned for heat [12]. Additional value could arise from offsetting costs of kudzu control, as income from bioethanol production could pay for kudzu eradication and land rehabilitation.

An important consideration is the sustainability of kudzu exploitation. The existing mass of kudzu has accumulated over years, and could presumably support a significant amount of bioethanol production in the near term; however, this standing stock would likely be exhausted should kudzu roots prove to be valuable. Sustained kudzu production would require either cultivation or allowing kudzu to re-establish on harvested landscapes. Cultivation of kudzu in North America would require amendments to existing regulations that list kudzu as a noxious weed and therefore ban its cultivation [23]. Because of its weediness, cultivation would also require careful management to prevent further damage to regional biodiversity and water quality. Without eradication efforts following harvest, kudzu will readily re-establish, as it propagates from root crowns and stem fragments [1,4]. It is probable that kudzu production could be sustained over the long term by this natural re-growth, although it is too early to estimate production values since the growth rate of kudzu roots is unknown.

In conclusion, kudzu-infested landscapes could be a valuable new source of carbohydrate for bioethanol production. To be exploited, however, additional research is required to resolve current uncertainties, notably, how much ethanol could be produced by a hectare of kudzu roots, and whether kudzu could be economically harvested. Harvesting and processing techniques have to be developed, and the area of harvestable kudzu should be assessed. Regulatory issues will have to be resolved, particularly if kudzu is to become a renewable resource. Because existing kudzu has already produced a large reservoir of carbohydrate on the landscape, we feel there is sufficient justification to warrant further research and the establishment of pilot projects to examine the feasibility of using kudzu for biofuel production.

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REFERENCES

[1] Forseth IN, Innis AF. Kudzu (*Pueraria montana*): history, physiology, and ecology combine to make a major ecosystem threat. Critical Reviews in Plant Sciences 2004;23:401–13.

[2] Blaustein RJ. Kudzu's invasion into southern United States life and culture. In: McNeely JA, editor. The great reshuffling: human dimensions of invasive alien species. Cambridge, UK: IUCN–The World Conservation Union; 2001.

[3] Global Species Invasive Database. 100 of the world's worst weeds. Auckland NZ: IUCN—The World Conservation Union, Invasive Species Specialist Group, 2007. 〈http://www.invasivespecies.net/database/species/〉.

[4] Wechsler NR. Growth and physiological characterisitics of kudzu, *Pueraria lobata* (Willd.) Ohwi, in relation to its competitive success. MSc thesis, University of Georgia, Athens, 1977.

[5] Mitich LW. KUDZU [*Pueraria lobata* (Willd.) Ohwi]. Weed Technology 2000;14:231–5.

[6] Sharkey TD, Loreto F. Water-stress, temperature, and light effects on the capacity for isoprene emission and photosynthesis of kudzu leaves. Oecologia 1993;95:328–33.

[7] Shurtleff W, Aoyagi A. The book of kudzu. Wayne, New Jersey: Avery Publishing; 1985.

[8] Miller JH, Edwards B. Kudzu: where did it come from? And how can we stop it? Southern Journal of Applied Forestry 1983;7:165–9.

[9] Bodner CC, Hymowitz T. Ethnobotany of *Pueraria* species. In: Keung WM, editor. Pueraria: the genus Pueraria. New York: Taylor and Francis; 2002.

[10] Tanner RD, Hussain SS. Kudzu (*Pueraria lobata*) root starch as a substrate for the lysine-enriched baker's yeast and ethanol fermentation process. Agriculture and Food Chemistry 1979;27:22–7.

[11] Parks LJ, Tanner RD, Prokpo A. Kudzu, a valuable potential commercial resource: food, paper and chemicals. In: Keung WM, editor. Pueraria: the genus Pueraria. New York: Taylor and Francis; 2002.

[12] Tanner RD, Hussain SS, Hamilton LA, Wolf FT. Kudzu (*Pueraria lobata*): potential agricultural and industrial resource. Economic Botany 1979;33:400–12.

[13] RFA (Renewable Fuels Association). Industry statistics. Washington, DC, USA: Renewable Fuels Association; 2007 〈http://www.ethanolrfa.org/industry/statistics/〉.

[14] Runge CF, Senauer B. How biofuels could starve the poor. Foreign Affairs 2007;86:41.

[15] DOE (United States Department of Energy). Breaking the biological barriers to cellulosic ethanol, a joint research agenda. Washington: United States Department of Energy, Office of Science; 2006 DOES/SC-0095.

[16] Solomon BD, Barnes JR, Halvorsen KE. Grain and cellulosic ethanol: history, economics and energy policy. Biomass and Bioenergy 2007;31:416–25.

[17] Sicher RC, Harris WG, Kremer DF, Chatterton NJ. Effects of shortened daylength upon translocation and starch accumulation by maize, wheat and pangola grass leaves. Canadian Journal of Botany 1982;60:1304–9.

[18] Hendrix DL. Rapid extraction and analysis of nonstructural carbohydrates in plant tissues. Crop Science 1993;33:1306–11.

[19] El Bassam N. Energy plant species: their use and impact on environment and development. London: James and James Ltd; 1988.

[20] Patzek TW, Pimental D. Thermodynamics of energy production from biomass. Critical Reviews in Plant Sciences 2005;24:327–64.

[21] Patzek TW. Thermodynamics of the corn-ethanol biofuel cycle. Critical Reviews in Plant Sciences 2004;23:519–67.

[22] Achremowicz B, Tanner RD, Prokop A. The effect of frost on the starch yield from kudzu (*Pueraria lobata*) roots of various ages grown in northern Alabama. Bioresources Technology 1994;47:149–51.

[23] National Invasive Species Information Center, United States Department of Agriculture, 2008; 〈http://www.invasivespeciesinfo.gov/plants/kudzu.shtml〉.