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ORIGINAL ARTICLE

Forest resources for second generation biofuel production

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Abstract
The gradual reduction in world oil reserves is increasing the importance of biofuel. First generation biofuels resulted in yields that are lower than general energy needs. At least in some cases, more energy was used for production than was obtained from the final fuel. Conflicts with food cultivation and high environmental impact are other possible drawbacks. Nevertheless, the multiple uses of plant lipids (fuels, food, many industrial uses) make fuel crops important cultivations. Moreover, cultivation of oil crops used for fuel only is probably economically advantageous for local needs and local economic cycles. Second generation biofuels should perform better in terms of yield and soil use. Among them, lignocellulosic biomass appears to be promising owing to its high available quantity and productivity. In particular, forest biomass (of cultivated trees) could potentially provide a large amount of biomass for biofuels. A general account on lignocellulosic biomass transformation to liquid biofuels (ethanol) is provided together with a quantitative example which calculates the potential biofuel production obtainable by cultivating forest biomass in abandoned and marginal land of Tuscany (Italy). Looking at official available data on energy demand in Tuscany, 15% of the current gasoline consumption could be replaced by such biofuel sources.

Keywords: *ethanol, forest biomass, lignocellulosic biomass, lignocellulosic biomass processing, second generation biofuels, Tuscany.*

Introduction

Petroleum-derived fuels are the most developed energy source, but raise many doubts regarding issues related to environmental sustainability and economy. The reduction of world oil reserves is destined to cause an increase in price (Kilian et al., 2009; Korpela, 2006; Saxena et al., 2009). Moreover, fuels derived from the combustion of fossil oil are the main cause of the rise in atmospheric carbon dioxide (CO_2) concentration, which is considered to be the main reason for climate change (e.g. Allen et al., 2009; Solomon et al., 2009). Global warming has led to the adoption, by more than 100 countries, of mitigation efforts to reduce the risks, impacts and damage due to climate change (Meinshausen et al., 2009; Pachauri & Reisinger, 2007).

Biofuels are fuels obtained through biomass processing. First generation biofuels are those obtained either directly from plant lipids or through fermentation of carbohydrate biomass, typically ethanol obtained by cereals. A list of first generation biofuels is reported in Table I.

The use of biofuels would reduce fossil fuel dependence since biofuels are renewable resources and have, after combustion, a theoretical net null CO_2 emission. (Nevertheless, these data depend on the energy costs deriving from cultivation and crude biomass processing.)

However, biofuels have some weak points: conflict with food agriculture with possible increases in food price, insufficient amount of land for cultivation to cover current energy needs, environmental impact depending on cultivation modes linked to the use of agrochemicals, and forest cutting for obtaining new land for energy agriculture use. Moreover, at least in some cases, the energy return on energy invested (EROI) would be too low to invest in biofuels (Scharlemann & Laurance, 2008).

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Table I. First generation biofuels.

Type	Use	Processing	Involved plants
Plant-derived oil (lipids)	Diesel engine fuel	None	Canola, oil palm, sunflower, soya, peanut and others
Biodiesel	Diesel engine fuel	Transesterification with methanol or ethanol	Same as above
Bioethanol	Benzine engine fuel	Fermentation of starch and other carbohydrates and distillation	Maize, sugarcane, sorghum, other cereals, cassava
Biogas (methane and other hydrocarbons)	Benzine engine, gas engine, turbines	Bacterial degradation and physical compression of derived gas	Organic waste

Nevertheless, the multiple uses of plant lipids (fuels, food, many industrial uses) make these crops important cultivations, while their cultivation for fuel only will probably be economically advantageous for local needs (i.e. for direct agricultural energy needs).

Second generation biofuels are not well defined as they represent a heterogeneous category. They are characterized by their theoretically overcoming some of the limitations of the first generation biofuels. Hence, they should have higher productivity and lower negative environmental impact than first generation biofuels. A partial classification of second generation biofuels is reported in Table II. The inclusion of some of these biofuels in the first or second generation categories may by debatable. According to Tilman et al. (2006), the second generation biofuels are those made from the breakdown of plant cellulose or lignin. Such biofuels could be produced from non-food plants such as prairie grasses or trees grown on marginal or degraded lands (Saxena et al., 2009). Even algae from aquaculture are to be included among second generation biofuels. Both microalgae (Schenk et al., 2008) and macroalgae (Aresta et al., 2005) can be used for this purpose. The advantage would be no competition with agricultural products and the higher photosynthetic efficiency of aquatic biomass compared with that of terrestrial biomass (Aresta et al., 2005; Chisti, 2007). Algae-derived fuels comprise lipids, carbohydrates and even directly hydrocarbons similar to petrol, e.g. *Botryococcus braunii* (Banerjee et al., 2002) and some dinoflagellates (Gallois, 1976).

Algal biomass could perform particularly well in terms of life cycle assessment (LCA), with the net energy gain being heavily influenced by the processing technology (Aresta et al., 2005).

Second generation biofuels from forest and crop residues, energy crops and municipal and construction waste would arguably reduce net carbon emission, increase energy efficiency and reduce energy dependency, potentially overcoming the limitations of first generation biofuels (Antizar-Ladislao & Turrion-Gomez, 2008; Hill et al., 2006).

Forest resources for producing biofuels are prevalent in tropical countries where oil-producing trees are extensively cultivated. The oil palm (*Elaeis guineensis*, Arecaceae) (Hartley, 1977) in particular, and some other more recently introduced species, e.g. *Jatropha curcas* (Euphorbiaceae), are included among the first generation biofuels. The cultivation of such plants is causing damage to the natural environment, mainly due to forest cutting or the use of natural arid environments in the case of *J. curcas*, while most of the produced oil is destined to be exported for the production of biodiesel or other oil-based industrial products in Europe and the USA.

One aim of this study is to evaluate the use of wood biomass as a second generation biofuel, and specifically the production of ethanol, which is the result of the enzymic degradation of the wood biomass. This biomass is composed mainly of lignin and cellulose. The theoretical productivity would be greater than with first generation biofuels (oil and cereal-derived ethanol) and at least some second generation biofuels appear particularly promising in

Table II. Second generation biofuels.

Type	Use	Processing	Involved plants
Lignocellulosic biomass	Benzine engine	Degradation of lignin and cellulose, fermentation, distillation	Forest biomass (poplar, willow and other), some fast growing grasses
Oil from algae	Diesel engine	Extraction, possibly transesterification	Cyanobacteria, unicellular eukaryotic algae, multicellular eukaryotic algae
Hydrocarbons from algae	Benzine and diesel engine	Refinery	Few species of unicellular eukaryotic algae, e.g. *Botryococcus braunii*, dinoflagellates
Carbohydrates from algae	Benzine engine	Degradation of wall carbohydrates, fermentation, distillation	Cyanobacteria, unicellular eukaryotic algae, multicellular eukaryotic algae

terms of their benefits and costs for biofuel production (Scharlemann & Laurance, 2008; Yu & Zhang, 2004). Moreover, second generation biofuels produced from non-food plants may perform better in environmental terms (Scharlemann & Laurance, 2008).

Zah et al. (2007) proposed a new conceptual scheme for the evaluation of different biofuels using just two criteria: greenhouse gas emissions and the overall environmental impact. However, they excluded from their analysis the second generation biofuels, such as those made from the breakdown of plant cellulose and/or lignin, because of insufficient data. Further investigations are needed in this field and a correct assessment should aim to analyse the environmental impact in the area of cultivation, which may be mountainous areas of high conservation interest.

Feedstock/raw material for lignocellulosic biofuels and biomass processing

Lignocellulosic biofuels are obtained from plants whose main biomass constitutes the residuals of plant cell walls (cellulose, hemicellulose, pectic substances) and woody parts of the plant such as tracheids, vessels and sclerenchymatic cells, whose secondary walls are impregnated with lignin.

The plants used most often for production of lignocellulosic raw material are (limited to species and genera most used in temperate countries): perennial C4 grasses such as *Miscanthus* × *giganteus* (Gomez et al., 2008) and *Panicum virgatum* (switchgrass) (Keshwani & Cheng, 2009), various species of poplar (genus *Populus* L. sp.) and willow (genus *Salix* L. sp.) (Salicaceae). The forest resources most often used for biofuel production are reported in Table III.

Potentially, waste derived from food crop production, e.g. rapeseed (Zabaniotou, 2008), textile crop residuals, e.g. cotton stalks (Shi et al., 2009), and the wood products industry, e.g. from paper mills, sawmills and furniture manufacture (Lin & Tanaka, 2006), could also be used as sources of lignocellulosic biomass.

Cellulose and lignin are the most abundant organic resources in the world, with 7×10^{11} t of cellulose being in existence at any time, with an annual turnover rate of 4×10^{10} t (Coughlan, 1985).

The degradation of these polymers is a natural process occurring in all forest soils and is carried out mainly by fungi (see Table IV for a partial list with references).

The initial conversion of lignocellulosic biomass into liquid fuel is the key step that limits the cost-effectiveness of the process. The carbohydrate polymers are tightly bound to lignin mainly by hydrogen bonds but also by some covalent bonds; the delignification of lignocellulosic raw materials is the rate-limiting and most difficult task to solve (Lee, 1997; Lin & Tanaka, 2006). The conversion of biomass into sugars is a key bottleneck in the process of biofuel production (Lee et al., 2008).

The biomass is initially processed using various systems for obtaining hydrolysis to produce simpler compounds. This process may include high temperature, acid treatment and/or high pressure. Recent developments in lignocellulosic biomass pretreatment have been reviewed by Hendriks and Zeeman (2009). The slurried material is fermented to produce alcohol, which is then purified by distillation and/or filtration to produce the desired fuel-grade quality ethanol (Lin & Tanaka, 2006).

Cellulose degradation is achieved with the help of fungi, using a series of specific enzymes (cellulases), initially to solubilize cellulose crystals (C1 cellulase), while other cellulases convert soluble cellulose to monosaccharides (Blain, 1975; Garraway & Evans, 1984). Hemicelluloses are heterogeneous polymers, with xylans being the most common form in wood (Cooke & Whipps, 1993). A range of enzymes is necessary to degrade hemicelluloses completely, such as the xylanases of the yeast *Cryptococcus albidus* (Cooke & Whipps, 1993). The pectic substances consist mainly of D-galacturonic acid monomeres linked by α-1,4-glycosidic links. To degrade these substances specific enzymes are also necessary, such as fungal pectinolytic enzymes (Blain, 1975).

Lignin is the most complex of the plant polymers. It is the second most abundant biopolymer after cellulose (Garraway & Evans, 1984), and is formed by branched polymers of three substituted alcohols: *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol. The white-rot fungi (Ascomicotina,

Table III. Forest resources for second generation biofuels.

Species	Vernacular name	Ecology/geography	Reference
Salix L. sp. (family Salicaceae)	Willow	Heterogeneous genus of mainly boreal trees and shrubs	Keoleian & Volk (2005)
Populus L. sp. (family Salicaceae)	Poplar	Genus of mainly boreal trees	Keoleian & Volk (2005)
Eucalyptus L'Her. sp. (family Myrtaceae)	Eucalyptus	Genus of warm climates	Keoleian & Volk (2005)
Pinus L. (family Pinaceae), gymnosperms	Pine	Various climates	Yuan et al. (2009)

Table IV. Microorganisms for degradation of lignocellulosic feedstock.

Species/group	Involved enzymes	Action	References
Various fungi	C1 cellulase	Solubilization of cellulose crystals	Blain (1975); Garraway & Evans (1984)
Various fungi	Other cellulases	Conversion of soluble cellulose to monosaccharides	Blain (1975); Garraway & Evans (1984)
Cryptococcus albidus and other species	Xylanases	Hemicelluloses degradation to monosaccharides	Cooke & Whipps (1993)
Various fungi	Pectinolytic enzymes	Pectic substances degradation mainly to D-galacturonic acid monomeres	Blain (1975)
White-rot fungi (Ascomicotina, Xylariaceae)	Laccases and peroxidases	Lignin degradation to a variety of phenolic compounds	Cooke & Whipps (1993); Kavanagh (2005)
Brown-rot (Basidiomycotina) and soft-rot (Ascomycotina, in wet wood)		Lignin degradation, but these fungi can use only cellulose and hemicellulose components of wood freed by lignin degradation	Kavanagh (2005)

Xylariaceae) can degrade these compounds and use them as the main source of carbon (Cooke & Whipps, 1993; Kavanagh, 2005), employing a cocktail of oxidative and peroxidative enzymes to attack the lignin polymers. Brown-rot (Basidiomycotina) and soft-rot (Ascomycotina, in wet wood) fungi can degrade only the cellulose and hemicellulose components of wood (Kavanagh, 2005). The degradation products of lignin are a variety of phenolic compounds (Kavanagh, 2005). A partial list of microorganisms used for the conversion of lignocellulosic-derived monomers to biofuels is given in Table V.

Nearly all ethanol fuel ethanol is currently produced by the fermentation of corn glucose in the USA or sucrose in Brazil (Lin & Tanaka, 2006). The raw materials used for the manufacture of ethanol for fermentation are classified into three main types of raw materials: sugars, starches and cellulose materials. Sugars can be converted into ethanol directly. Starches (from corn, cassava, potatoes and root crops) must first be hydrolysed to fermentable sugars by the action of enzymes from malt or moulds. Cellulose (from wood, agricultural residues, waste sulphite liquor from pulp, and paper mills) must likewise be converted into sugars, at present generally by the action of mineral acids. Once simple sugars have formed, enzymes from microorganisms can ferment them to ethanol (Lin & Tanaka, 2006).

Direct transformation of cellulose to ethanol can be conducted with the help of anaerobic thermophilic bacteria, such as *Clostridium thermocellum* (Herrero & Gomez, 1980; Zertuche & Zall, 1982), as well as some filamentous fungi, including *Neurospora* sp. (Yamauchi et al., 1989), *Aspergillus* sp. (Ito et al., 1990), *Trichoderma viride* (Lin & Tanaka, 2006) and other species. The process carried out by these microorganisms is slow and with a poor yield (0.8–60 g l^{-1} ethanol), owing to the low resistance of microorganisms to higher concentrations of ethanol (Lin & Tanaka, 2006). Moreover, and particularly in the case of bacterial fermentation, the fermentation produces various by-products, e.g. acetic and lactic acids (Herrero & Gomez, 1980; Wu et al., 1986).

Fermentation of lignin cannot be carried out by most yeasts. A previous hydrolysation is hence necessary to obtain monomers (Sun & Cheng, 2002). Subsequent use of these monomers as fuels is not straightforward since their composition is variable.

Plants producing ethanol from cellulose have been active in Canada since 2004 (Tampier et al., 2004),

Table V. Microorganisms for fermentation of monomers produced by lignocellulosic material degradation.

Species/group	Involved enzymes	Action	Reference
Yeasts (*Saccharomyces cerevisiae*)	Enzymes of fermentation pathways	Fermentation of carbohydrate monomers to ethanol	Lin & Tanaka (2006)
Bacteria (e.g. *Zymomonas mobilis*, engineered *Escherichia coli*)	Enzymes of fermentation pathways	Fermentation of carbohydrate monomers to ethanol	Lin & Tanaka (2006)
Cellulose-fermenting bacteria (e.g. *Clostridium thermocellum*)		Fermentation of cellulose to ethanol	Lin & Tanaka (2006)
Cellulose-fermenting filamentous fungi (e.g. *Monilia* sp., *Neurospora crassa*, *Trichoderma viride* and other species)		Fermentation of cellulose to ethanol	Lin & Tanaka (2006)

while other pilot plants are being developed in many countries (Lin & Tanaka, 2006).

Forest products for lignocellulosic biofuels

Forests contribute ±50% of terrestrial net primary production (Bonan, 2008) and hence the use of forest products as a source of lignocellulosic biomass for biofuels seems to be very promising. Some researchers have calculated that short-rotation woody crops will be the single most important source of biomass in the coming years (Berndes et al., 2003), while the use of natural forest biomass for energy production would be detrimental to the biodiversity of those ecosystems.

The most frequently cultivated woody trees in temperate countries are willows, poplars and potentially pine (genus *Pinus*) (Yuan et al., 2009). Eucalyptus (*Eucalyptus* spp., particularly *E. grandis* and *E. saligna*) is a model species for warmer climates (Keoleian & Volk, 2005; Saxena et al., 2009) and *Leucaena leucocephala* (Fabaceae) is a recently investigated nitrogen-fixing alternative (Saxena et al., 2009; Carroll & Somerville, 2009).

Willows and poplars are normally found at low altitudes. This is well known for poplars: *Populus alba* L., *P. nigra* L. and *P. canadensis* L. (of hybrid origin), but the eurosibiric *P. tremula* L. grows also at 2000 m altitude. The whole genome sequencing project of *P. trichocarpa* may lead to genetic modifications of species to increase their productivity or adaptability to different environmental situations. The genus *Salix* shows a high level of biodiversity at the species level, with hundreds of known hybrids in the Italian peninsula and even some mountain dwarf representatives. With such natural biodiversity it is possible to find species adaptable to marginal (from an agricultural point of view) territories, such as *S. pentandra* L. and representatives of the *S. nigricans*, *S. caprea* and *S. purpurea* groups. The high generic variability in different geographical areas leads to a search for cultivars highly adapted to different types of marginal territories.

Known species of willow cultivated as energy crops are *Salix alba* and *S. viminalis*; while poplar species comprise *Populus nigra, P. euramericana cv. Robusta, P. alba, P. tremula, P. balsamiferas, P. maximowiczii, P. tomentosa and P. euphraetica* (Fischer et al., 2005).

The advantages of willow are linked to its ability to attain high biomass production levels in just a few years and to reach its maximum annual increment rapidly, along with a good tolerance of high planting densities and its rapid growth rate following coppicing (Keoleian & Volk, 2005).

Environmental impact assessment and a case study: potential of forest-derived biofuels in Tuscany

The use of natural areas for the production of lignocellulosic biomass would have a high environmental impact on biodiversity in temperate countries, as it has in some tropical areas because of oil palm cultivation. The negative impact on the natural environment may be reduced by cultivating trees on marginal land, or on land abandoned by agriculture. A large amount of land has been severely degraded, to the point that it could be considered abandoned agricultural fields. Field et al. (2008) estimated that abandoned lands have reached approximately 450 Mha worldwide.

How much abandoned and marginal land is available in Europe? The evaluation of this quantity is very complex but, fortunately, some data are available from the statistics offices of many local governments, such as in Tuscany (central Italy).

The PIER (Piano Indirizzo Energetico Regionale: Regional Energy Plan, 2008, p. 104) of Tuscany showed that energy demand increased during the period 1995–2003 from 8027 ktep (ktep = thousands of tonnes-equivalent of petroleum) (2495 for transport) to 8987 ktep (2831 for transport). Half of this total energy consumption was derived from petroleum-derived fuels.

According to the Forest Inventory of Tuscany for 1998 (Various Authors, 1998), a total of 1,086,016 ha of forest existed in Tuscany (total surface area 2,299,700 ha) in that year. Based on the state report for the forests in Tuscany 2007 (ARSIA, 2008) there was a total of 1,151,539 ha of forest. The numbers reflect an increase of 65,523 ha over a period of 9 years. This alteration in forest land size could be attributed to the increase in marginal and abandoned land resulting from the reduction in income obtained from traditional agricultural practices in these unfavourable areas. Most of those areas are normally located at higher altitudes (even if less than 1000 m) or in areas far from communication routes.

What is the potential productivity of such areas using willow or poplar, the best known species for this purpose? A reasonable productivity for lignocellulosic forest biomass in such conditions would be 10 tdm ha^{-1} $year^{-1}$ (6.21–16.9 tdm ha^{-1} $year^{-1}$, where tdm = tonnes of dry mass) for willow and poplar (Labrecque & Teodorescu, 2005). The energy obtainable from such a quantity would be: 65,623 ha × 10 tdm ha^{-1} $year^{-1}$, with a conversion factor for converting lignocellulose to ethanol: 0.26 tep for tdm (where tep = tonnes equivalent of petroleum) (Lee et al., 2008, supplemental online

material, Table S1), totalling about 170,620 tep (=7,166,040 GJ of energy).

Considering a need of gasoline for families in Tuscany of 1125 ktep (kilotonnes equivalent of petroleum) in 2005 (Table VI, approximately unchanged in 2009, owing to the recent reduction in mobility with the current general economic crisis), the energy production obtainable by biomass in recently abandoned land may substitute 15.2% of the current (2005) gasoline consumption by families in Tuscany.

The EROI can be evaluated from LCA methodology, which provides a comprehensive systems-based analysis of the energy and environmental performance of a product system (ISO, 1997). In LCA, the material and energy inputs and outputs are quantified throughout the lifetime of a product, from raw material acquisition through production, use and disposal. Potential environmental impacts of the product system are then assessed based on its life-cycle inventory.

A preliminary evaluation can be proposed based on a previous assessment (Keoleian & Volk, 2005). The final evaluation will take into account cultivation methods and local transformation and use of raw material in local conditions.

The net energy ratio (harvested biomass energy at the farm gate divided by fossil energy consumed in production) for agricultural production of willow biomass after the first rotation was 16.6. This ratio increased to 55.3 when considering output and consumption over a period of seven rotations. In other words, according to Keoleian and Volk's model, 55 units of energy stored in biomass were produced using one unit of fossil fuel energy. Moreover, greenhouse gas production was reduced by 95% compared with fossil fuels (Keoleian & Volk, 2005).

These preliminary calculations should take into account that potential productivity in lignocellulosic biomass could reach over 15 tdm ha^{-1} $year^{-1}$ on suitable land (Fischer et al., 2005), while in marginal or abandoned (and hence less suitable) land productivity would be lower. Further investigations are needed to select willow and poplar varieties adapted to these less favourable conditions to maximize the productivity.

A forestry model for poplar predicts yields of 12.4 t ha^{-1} on non-irrigated, unfertilized land and 22.5 t ha^{-1} on irrigated, fertilized land, while a study in Quebec found yields of 17.3 t ha^{-1} for poplar and 16.9 t ha^{-1} for willow, another promising candidate, without any use of fertilizer or irrigation (Carroll & Somerville, 2009).

Other general suggestions are related to the localization of processing plants, which should be as close as possible to biomass sources. This would reduce the necessity for biomass translocation (and related transport energy waste) to increase process EROI.

The possible use of waste compost (from organic waste) obtained from the collection of various wastes could also be considered to be used for the process, since the use of waste-derived organic fertilizer faces difficulties regarding food production, at least in Italy.

Biotechnological breakthroughs that could increase the yield of lignocellulosic biomass processing have been reviewed by Yuan et al. (2009). The immediate factor impeding the emergence of an industry converting cellulosic biomass into liquid fuels on a large scale is the high cost of the process, rather than the cost or availability of feedstock (Lee et al., 2008). This is why efforts to reduce costs are necessary, particularly for raw material transportation and the enzymes (or enzyme-producing microorganisms) that are used for the degradation of lignocellulosic biomass.

Conclusions

Lignocellulosic biomass (assigned to second generation biofuels) has a much higher productivity than

Table VI. Total energy demand of a family in Tuscany (ktep).

Year	1995	2000	2001	2002	2003	2005	2010[a]	2012[a]
Solid	34	56	67	61	63	66	71	73
Liquid	1598	1612	1580	1498	1445	1510	1630	1673
Of which diesel	285	242	264	251	257	269	290	298
Of which gasoline	1172	1188	1176	1112	1077	1125	1215	1247
Gaseous	1082	1036	1118	1103	1139	1191	1286	1320
Of which methane	1082	1036	1118	1103	1139	1191	1286	1320
Electric energy	316	337	334	346	361	377	407	418
Total per family	3030	3041	3100	3007	3008	3144	3394	3484

Note: Source: IRPET on ENEA data. IRPET: Instituto Regionale Programmazione Economica Toscana; ENEA: Italian National Agency for New Technologies Energy and Sustainable Economic Development.
[a]IRPET estimate.
ktep = thousand tonnes equivalent of petroleum.

traditional biofuel cultivation. The possibility of producing lignocellulosic biomass from cultivations in marginal and abandoned land could be of interest for biofuel production. Current disadvantages are related to the costs of biomass processing to obtain bioethanol. A preliminary evaluation on the use of abandoned (in the last 10 years) land for lignocellulosic biomass production in Tuscany indicated that such land could produce up to 15% of currently used transport fuels, even with current transformation technologies. The process could be made more convenient by using organic waste-derived compost fertilizers produced *in situ* (in Tuscan cities) and by using biomass processing plants located as close as possible to productive short-rotation woody crop plantations.

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