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CONVENTION ON THE CONSERVATION OF EUROPEAN WILDLIFE
AND NATURAL HABITATS

Standing Committee

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**Invasiveness of biofuel crops
and potential harm
to natural habitats and native species**

FINAL VERSION

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ABSTRACT

Several factors prompted the development of biofuel cropping within agroecosystems. These include a growth in population and energy demand, geopolitical instability linked to demand for fossil fuels, concerns about global warming and calls for 'carbon neutral' energy. In anthropogenic manipulated agroecosystem, many factors multiply the creation of newly available niches. Consequently, the occurrence and establishment of invasive alien species with the potential to spread and cause harm, or constrain elements of semi-natural habitat or vegetation remnants may increase. The invasiveness of weedy germplasm may also be accelerated by the presence of cultivated species, escaping from fields through crop movement or on livestock. This risk of invasion is likely to rise in the future use of agricultural land for widespread and intensive cultivation of crops for energy production. In fact, in the recent past, many government reports, including the Convention on Biological Diversity and scientific literature have highlighted the potential impact that biofuel crops may have on natural, semi-natural and agriculture ecosystems. The characteristics of energy crop species, of their habitats, of cropping systems and of farm subsidies are a “weedy merging combination” that could transform farmland into a source of new invasive species that may spread into vegetation remnant, ultimately harming the functionality and biodiversity within agroecosystems. Being the crop escape a consequence of the farming system a precautionary principle must be taken into account even if no immediate evidence of harm to native habitats is present.

INTRODUCTION

Increased population growth, greater energy demand in industrialised countries, geopolitical instability due to the fossil fuel market, air pollution, concerns about global warming and the need for “carbon neutral” energy have all enhanced the development of biofuel cropping systems (agriculture and forestry), that is the use of plant biomass for the production of energy. In the EU, in fact, more than 80% of greenhouse gas emissions are related to the production of electricity, heat and road transportation (EEA 2008). One of the main energy policy targets of the European Union is to increase renewable energy sources by 2010. Various laws have been passed to help achieve this target, for example, the Biofuel Directive (2003), the Biomass Action Plan (2005) and the Biomass Strategy (2006), which promoted the use of biofuels for transport, heating and electricity, in the EU and developing countries, because of their positive effects on the environment by 2020.

In directive 2003/30 the European Commission (EC) set a goal of 5.75 % by 2010 for the use of biofuels for transportation. In 2009, the EU adopted a Directive (2009/28/EC) to promote the use of energy from renewable sources. This endorsed a mandatory 10% minimum target to be achieved by all Member States for the share of biofuels in transportation.

The Common Agricultural Policy (CAP) supports both the supply of bioenergy from agriculture and forestry as well as the use of bioenergy on farms and in rural areas with direct incentives for the production of raw plant material. The EU grants an energy crop premium of 45 EUR/ha on a maximum 2.0 million ha of eligible land and allows farmers to grow energy plants on set-aside land.

Farmers, since 2004 have been much more likely to produce energy crops, when subsidies were introduced as part of the reformed CAP to stimulate the European biofuels sector. In 2007 the area of energy crops declared eligible for the subsidy exceeded approximately 42%. In Europe, States and local government policies promote the use of crops and short rotation forestry as a source of energy, also through different rural, industrial and environment support Plans. There is, however, growing concern about the potential harm that new, intensive and extended cultivation could have on the territory.

To gain socio-economic and environmental benefits, biofuel crops need to be farmed in an environmentally sustainable manner. There are now great apprehensions about food security, loss of soil fertility and land clearing. Loss of biodiversity is another key concern, linked to competition between escaped aggressive crops cultivars with native species.

Directive 2009/28/EC sets sustainability criteria for biofuels production. These standards relate to biodiversity, protection of rare, threatened or endangered species and ecosystems. It calls on countries

to monitor the impact of biomass cultivation, such as through land use changes, including displacement and introduction of invasive alien species.

Several of the proposed biofuel crops are, in fact, already considered invasive “elsewhere”. Still, a precautionary principle should apply, even if there is no immediate evidence of crop escape. It should be noted that newly introduced species may take a long time, after plantation, to escape, spread and establish themselves and may irreversibly harm the natural habitat.

The spread of invasive alien species within the European Union is a great and growing concern. Priority has been given to weed control and eradication in both the Biodiversity Action Plan and the Common Agricultural Policy, alongside the development of early warning systems and optimising the use of available measures. The CoE's Bern Convention and EU Directives ‘Habitats’ and ‘Bird’ recommend that member States control the introduction of non-native species and prevent accidental introductions. Under the LIFE financial instruments and the several Framework Programmes for Research and Technological Development, the European Community has invested heavily in this field in recent years, setting up numerous projects to tackle Invasive Alien Species (IAS). Importantly, management action plans were granted more funding than research activities (Scalera 2009).

Invasive Species (IS) have a great effect on the loss of functionality and biodiversity in agroecosystems considering, in particular, the future use of agricultural land for widespread and intensive cultivation of energy crops, for bioenergy production and carbon sequestration. Many of the proposed and used crop species have in fact “invasive characteristics”. The recent Conference of the Parties to the Convention on Biological Diversity (COP 9), held in Germany in May 2008, adopted decisions regarding agricultural biodiversity and sustainable production of biofuels, and encouraged Parties to the Convention to provide risk assessment procedures for the spread and effective management of IAS.

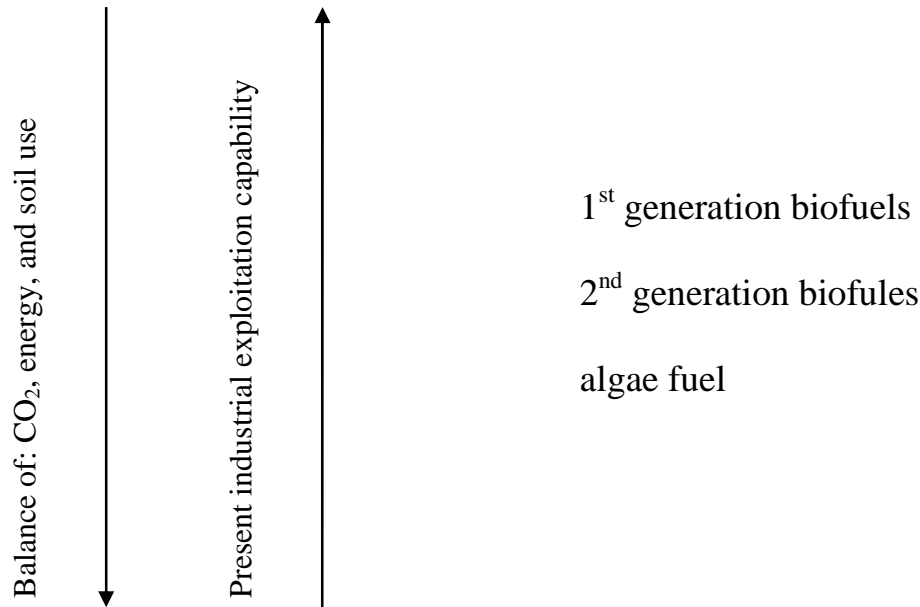
Within the European Union, many Directives, Reports and Communications (EC 2003, COM 2006, EEA 2007, COM 2008) highlight the need for each country to develop a national strategy and determine management priorities for alien species. In the EU's Common Agricultural Policy, national strategies for weed control and eradication feature both in the first pillar (Cross-compliance: Good Agricultural and Environmental Conditions) and in the second pillar (axis 2 Land management and Environment conservation).

The presence of invasive plant species within agro-ecosystems may damage both the functionality of planned biodiversity and the conservation of associated biodiversity (Vandermeer, et al. 2007). Invasive plant species within agroecosystems may harm both farmland functionality (i.e. obstructing ditch banks and river channels or reducing the harvest yield) and biodiversity (i.e. harming native hedgerows, semi-natural and remnant vegetation, native habitats surrounding agricultural areas). They also tend to slow down or “stuck” (*sensu* Hobbs and Cramer 2007) the secondary succession re-naturalization processes of abandoned fields. In sustainable agriculture productions, the functionality of the field margins is associated to rich biological communities with beneficial species including crop pollinators and pest predators (Marshall 2004). The escape, and consequent spread, of invasive crops from agriculture fields will diminish the functioning of these edges.

BIOFUELS

Solar energy is stored in sugars via photosynthesis and then in a variety of secondary products that can be converted to fuel.

Biofuel: Energy from plant materials. This can be summarized as follows:



- 1st generation biofuel produces: ethanol from sugars or starches; biodiesel from vegetable oils such as nuts and seeds; heat from combustion of solid, dry phytomass. 1st generation includes most of the species currently harvested for food and forestry production.
- 2nd generation biofuel produces: synthetic fuel and fuel ethanol from cellulosic material. The production processes can use a variety of non-food crops.
- Algae fuel: micro algae can produce H₂ and oil from lipids. To efficiently gain energy from algae, many research institutes focus on molecular genetics research, engineering and mutants creation. (box 1)

There is currently no large scale industrial exploitation of 2nd generation biofuels and algae, even though they represent a better balance of CO₂, energy, and use of soil.

At present, the technology is not efficient enough and there is a lack of broad expertise.

THE BIOFUEL WEEDY COMBINATION

a) The Biofuel Species Invasiveness Traits

Several biofuel crops, which many countries promote as alternatives to fossil fuels, share traits with invasive species (Raghu et al. 2006; Crosti & Forconi 2006; Low & Booth 2007; Howard & Ziller 2008; Barney and DiTomaso 2008, Buddenhagen et al. 2009) including broad ecological amplitude, rapid growth, high seed production, common occurrence of vegetative spread, and resistance to pests and diseases. Some biofuel crops have the potential to escape from cultivation, given that they are “selected” mainly for their efficiency in seed set and phytomass production.

Artificial selection for agricultural and horticulturally advantageous traits, such as greater fertility, pest resistance, growth rates and geographical range may increase invasiveness (Kitajima et al. 2006). Crop domestication is considered one of the main pathways for biological invasions (Reichard and White 2001). In particular, according to Barney and DiTomaso (2008), biofuel feedstock are bred to thrive in conditions that mimic natural habitats (and not the artificially rich agronomic conditions of traditional food crops). This increases the likelihood of recruitment in natural habitats.

b) The Invasibility Of The Agroecosystem

Agroecosystems are particularly susceptible to plant invasions as both community invasibility and species invasiveness merge (*sensu* Richardson and Pyšek 2006). In agroecosystems, anthropogenic manipulation of land for agricultural production has greatly changed the original natural

ecosystem (Gliessman 2000). Factors contributing to the establishment of IS in new or temporarily “vacant niches” include: habitat modification or degradation, fragmentation, misuse of chemicals, distorted water balance and nutrient cycle, soil tillage, altered fire regimes and abandonment of arable lands. Agriculture may ease the spread of alien plants by modifying the natural environment, making it more susceptible to invasions (Hulme 2005). Farmlands are habitats prone to new introductions, plant naturalisation and invasion, and so may enable the spread of alien plants (Chytry et al. 2008). Propagules of crop species, which often have both sexual reproduction and vegetative growth, may also escape from cultivation and become established (Williamson 1996; Gordon and Thomas 1998; Daehler and Carino 1999; Mack 2000, Maillet and Zaragoza 2003). The spread of germplasm is also amplified by livestock, which can transfer seeds into uninfested areas, and by dispersal through movement of equipment and goods. Around the world (Hulme et al. 2008), and in Europe (Hulme 2007), most invasive alien species were introduced for agricultural and horticultural purposes. On this issue, the Council of Europe recently endorsed a “Code of conduct on horticulture invasive alien plants” (Heywood and Brunel 2008); this follows its promotion of a “European strategy on invasive alien species” (Genovesi and Shine 2004).

c) The Cropping System

The particular ‘cropping system’ for biofuels increases the probability of germplasm escape into the agroecosystem.

Sowing (or planting) massive quantities of vigorous plant varieties in repeated introductions, often supported by economic subsidies, in various climates and soil conditions increases the likelihood of “crop escape”, and subsequent establishment of new biological invaders. Large scale and frequent introductions alone increase the probability of invasion (Crawley et al. 1996).

Moreover, all species used for heat production (annual but also perennial species with an annual cropping system) are harvested at the end of the yearly life cycle, when the part above ground has lost most of its water content. This procedure is used because it makes it more efficient for the harvesting, packaging, storage and transport, also considering the fact that for high combustion performance efficiency the crops need to be desiccated before entering the combustion cell. This would, however, lead the plant to the production of seeds (and pollen) that may therefore increase the probability of crop escape due to natural dispersion during storage and unprotected transportation between fields or to energy conversion factories.

Agriculture subsidies may also increase the spread of biofuel species. Subsidies were introduced in 2004 (under reforms to the CAP) to stimulate the European biofuels sector, leading to greater incentives for farmers to cultivate energy crops.

The Common Agricultural Policy (CAP) supports the supply of bioenergy from agriculture and forestry and the crops should enter into the energy chain. In rural areas however, it is difficult to assess if there is a real link between biofuel crop subsidies and crop processing and/or transformation as farmers are generally paid for cultivated area more than for crop harvested (a similar process occurred after the CAP 2004 mid-term reform when farmers received payments based on historical entitlements instead of annual yield production-“decoupling”). This could lead to feed stock being left to rot in fields, thus increasing germplasm crop escape through seed natural dispersion and pollination.

Biofuel agronomic studies, aiming to increase biofuel traits (and consequent invasiveness potential of the taxon), are also financially supported by the EU framework (i.e “*Panicum virgatum*”).

THE ASSESSMENT

The combination of these factors (species invasivity, habitat invasibility and cropping system) increase the potential for naturalization and invasiveness of biofuel species.

For this reason, biofuel crops should be subject to risk assessments before cultivation.

Several countries have already adopted a Risk Assessment to identify high-risk species, thereby allowing decision-making on prevention and eradication, so as to avoid ecological and economic harm (Pimentel et al. 2005).

While the biology and ecology of weed species help to signal the potential for invasion, a specified Risk Assessment may help to determine which new crops should be considered potential pests and then regulated as such.

Many invasive plant risk systems have been developed for Central Europe (Weber & Gut 2004), North America (Reichard and Hamilton 1997), and by FAO (2005) for developing countries.

In particular, the IPPC and EPPO were involved in the development of a Pest Risk Analysis to evaluate the potential spread of a pest or disease for member States under the ISPM (International Standard for Phytosanitary Measures).

The AWRAS (Australian Weed Risk Assessment System), however, has been used for longer and more widely than any other predictive model for invasive plants (Gordon et al. 2008) and recently has been validated also for Mediterranean Europe (Gassò et al. 2009; Crosti et al. 2009).

The AWRAS worksheet application is based on 49 questions on several life history traits and (domestication/ cultivation, climate and distribution, weed elsewhere, undesirable traits, plant type, reproduction, dispersal mechanisms, persistence attributes) generates a numerical score relating to the invasive potential of that species. The outcome can be used to accept/reject the species for importation or as a management tool to identify which are the life history traits that more than other are responsible for invasiveness.

Assessment should be used as a screening tool for both preventing cultivation of new, or not broadly farmed, biofuel crop species that could become invasive.

It is particularly recommended for those regions that intend to introduce new cultivation crops for energy production. In fact, simply changing the climate and soil parameters, and assessing the invasiveness potential from cultivar selection, is enough to evaluate the invasiveness potential of alien species in a specific region.

Barney & DiTomaso (2008) for North America and Buddenhagen et al (2009) for Hawaii tested invasiveness of biofuel crop using an “adapted” AWRAS. Outcomes of both studies underlined the fact that biofuel crops are selected for the same traits considered successful in invasive species.

ISPRA (the Italian Institute for Environmental Protection and Research) adapted the AWRAS for Central Mediterranean Italy and screened 15 proposed biofuel species, showing that at least 9 have the potential to become invasive species; of these some are already recognised as weeds both in Italy and elsewhere in mediterranean-type regions while others are not yet broadly farmed (Table and photos)..

Species	WRA score	WRA outcome	2nd screening	established cropping system in Italy	Raunkiaer's life form	Family
<i>Acacia saligna</i>	12	rejected		no	P	Leguminosae
<i>Ailanthus altissima</i>	17	rejected		yes	P	Simaroubaceae
<i>Crambe abyssinica</i>	0	accepted		no	T	Brassicaceae
<i>Cynara cardunculus</i> var <i>altilis</i>	16	rejected		yes	H	Asteraceae
<i>Helianthus tuberosus</i>	12	rejected		yes	G	Asteraceae
<i>Hibiscus cannabinus</i>	5	evaluate	rejected	yes	H	Malvaceae
<i>Jatropha curcus</i>	15	rejected		no	P	Euphorbiaceae
<i>Kochia scoparia</i>	11	rejected		no	G	Amaranthaceae
<i>Melia azedarach</i>	12	rejected		no	P	Meliaceae
<i>Miscanthus sinensis</i>	11	rejected		no	T	Poaceae
<i>Panicum virgatum</i>	4	evaluate	rejected	no	P	Poaceae
<i>Paulownia tomentosa</i>	4	evaluate	further evaluation	yes	P	Bignomaceae
<i>Robinia pseudoacacia</i>	16	rejected		yes	P	Leguminosae
<i>Sorghum bicolor</i>	6	rejected		yes	T	Poaceae
<i>Zea mays</i>	-1	accepted		yes	T	Antropogonoideae

Table. Species proposed for biofuel cultivation, WRA scores and outcomes. Raunkiaer life forms: Ch= Chamaephytes; G= Geophytes; H= Hemicryptophyte; P= Phanerophyte; T= Therophytes. (Source ISPRA 2008 Crosti et al.)



Photo *Helianthus tuberosus* escaped from field but no harm to natural habitat



Photo *Acacia saligna* harming natural mediterranean sand dune ecosystem



Miscanthus x giganteus allopolyloid that does not produce viable seed (as shown in the photo it reproduces vegetatively).



Sweet sorghum cultivation in Tuscany. Surveys are undergoing to monitor germplasm spread in the surrounding natural areas.

THE MITIGATION

The potential adverse impacts of new biofuel crops or biofuel cropping systems should be balanced with the short-term commercial benefits (Barbier and Knowler 2006). Introductions that cause accidental damage to habitats and ecosystems could be significantly cut by minimizing invasive traits during horticultural breeding programs (Anderson et al. 2006) and by adopting cultivation criteria to mitigate the invasiveness of biofuel crops.

Until now research on biofuel trial plantations has mainly focused on biomass production and energy efficiency and has not assessed the risk of “crop escape”. Newly introduced species may also take a long time to escape, spread and establish themselves (Hobbs and Humphries 1995), existing as “sleeper weeds”; in Europe lag time (period between species introduction and the time when it begins to spread or invade) for woody plants which had escaped from cultivation was estimated at 170 years for trees and 131 years for shrubs (Kowarik 1995). Time lags of more than 50 years are often assumed to be common in plants (Hobbs and Humphries 1995). Invasive tropical plants have shorter lag times compared to temperate species (Daehler 2009) and this can explain, together with the change use of soil, why *Jatropha* in tropical ecosystems showed already evidence of escapes and invasions into natural ecosystems.

Some reports suggest using native (*sensu latu*), rather than alien, species to reduce habitat loss and biodiversity harm. It should be noted, however, that even native species that are dominant within particular habitats (in grasslands or in high stand forests) are not efficient in phytomass production. So to be harvested for biofuel production, most of the species “claimed” as native in cropping systems are, in fact, species with a wild relative, selected or domesticated for specific traits which confer, among the others, greater invasiveness capacity (annex 2).

Spread of “wild related” species into natural habitat may impact native biological diversity as they can compete with native vegetation and increase hybridization (impacting the genetic integrity) with congeneric native species. New hybrids, in addition, can compete better for resources with the other species of the native plant community.

Despite the large economic (and environmental) benefits of biofuel cultivations, appropriate cultivation criteria (in contrast to agronomic efficiency) and buffer zones should be introduced to limit the risk of species establishment outside of cultivation fields, especially nearby natural or semi-natural habitats reducing the capacity of seed production, seed dispersal, and presence of sites suitable for seed germination and survival (including pathways).

a) Limiting seed production and germination capability

The presence of a conspicuous seed set usually reduces the cost of crop establishment. For this reason a “good” crop produces a large amount of viable seeds. Many biofuel crops produce massive quantitative of viable seeds, furthermore alien species are sometimes better adapted to growth and reproduction in agroecological environment (Maillet & Lopez-Garzia 2000).

Therefore cultivation practices that reduce seed production need to be carried out for those species that may establish in the wild through seed dispersal. Practices may include:

- mechanical removal of inflorescences (i.e inflorescences cutting before stalk harvest for cardoon, or during coppicing for species used in SRF such as acacia, robinia and ailanthus);
- harvest before seed development;
- growing crops in conditions where seeds do not reach maturation/germination (i.e: *Kochia scoparia* does not produce flowers at average temperatures below 15 °C; Kenaf needs at least 60 days of no frost for seed development; *Panicum virgatum* requires precipitations in Spring);
- use of germplasm lines that reduces seed viability and germination or use of stable sterile genotypes (as suggested for willows and poplars in annex 3).

b) Limiting seed accidental dispersal

It is important to limit any escape of propagules during harvest, storage and transportation.

Machines and equipment need to be cleaned after use, there should be no storage in open fields, and transport trucks should be covered. In addition contact between grazing herbivores and invasive crops should be limited, in farmlands, in fact, seed spatial movement is increased by the great range of animal organism that are capable of dispersing seeds (Benvenuti 2007).

c) Limiting the connection between seeds and sites suitable for germination and seedling recruitment

To recruit, a species needs a site suitable for germination and seedling establishment. Therefore potentially invasive biofuel crops should not be cultivated close to sites that can act as source systems, stepping stones, or ecological corridors such as: abandoned land, disturbed areas or river banks. Crops, in fact, are often planted near rivers, and in case of invasive biofuel crops (photos) the water system acts as a pathway for propagules.



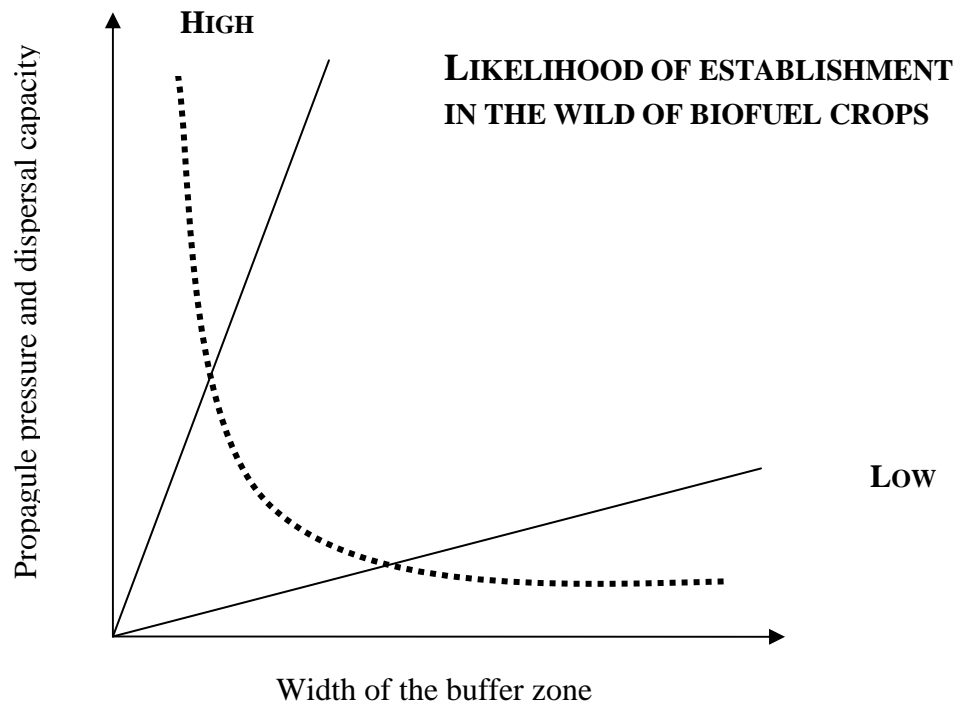
Photo *Ailanthus altissima* plantation nearby a river



Photo Poplar and ailanthus plantation nearby the river banks of the Danube River

Consequently between the crop field and natural vegetation (or ecological corridors which are “exceptional pathways”) a buffer zone should be established to act as a specific biological barrier for the invasive crop. Within farmlands, the buffer zone can be set with other (non-invasive) cultivations or through periodic soil ploughing. Buffer zones need to be specific (to reduce only the dispersion of

the undesired germplasm) and to be calibrated according to the invasiveness capacity of the crop. The area width should relate to the distance of dispersal of the species while the soil ploughing time should relate to the minimum regenerative time (from germination to production of viable seed, or the time taken for a vegetatively reproduced plant to duplicate itself). In case of *Ailanthus altissima* plantation, for example, a large buffer zone of 100 metres should be ploughed at least once every three years to reduce the likelihood of establishment of species. The probability of invasive crop species establishing in a natural habitat increases with the number of propagules and their dispersion capacity; it decreases according to the distance between the crop and the natural habitat (figure).



Paulownia tomentosa cultivation use as a feedstock for biofuel production



Ailanthus altissima weed “everywhere” and still proposed as energy crop

GOOD MANAGEMENT PROCEDURES

A “NON-EXHAUSTIVE” LIST OF “GOOD MANAGEMENT PROCEDURES” TO LIMIT THE POTENTIAL INVASIVENESS OF CERTAIN BIOFUEL SPECIES INCLUDES:

- use of pre-cultivation weed/pest risk assessment as a screening tool for each type of climate and soil type;
- reduce propagule dispersion during storage and transportation by adopting severe phytosanitary regulations (as if dealing with a pest);
- specific cultivation practices that limit seed production or use stable sterile genotypes;
- subsidy schemes strictly connected to crop harvesting/processing and not based on historical entitlements;
- dedicated studies on the auto-ecology of the planted species and pre-introduction field trials;
- landscape ecology assessment on: land use, disturbance events – such as fire or natural clearings – in nearby wild habitats, pathways and on the invasibility of the surrounding plant communities prior to localization of dedicated arable fields for biofuel crops;
- monitoring/surveillance sites located around biofuel fields, in habitats likely to be invaded (including ecological corridors/stepping stones or greenways) and in vegetation remnants, to provide an early warning of possible escape from crop fields;
- presence of a buffer zone between crop fields and natural habitats or ecological corridors (acting as pathways);
- indicating specific biological and chemical control techniques, updated by the EU herbicide review;
- identify local and regional stakeholders capable of rapidly eradicating and controlling invasive biofuel crops, via a coordinated program with local government Environmental Agencies;
- training and education on crop escape issues for farmland employees;
- target potential invasive biofuel crops to facilitate early warning and rapid response systems;
- biofuel investors should consider assurance bonds to insure the biofuel field against environmental damage due to crop escape; in case of damage, the bond should be used for environmental restoration.

ANNEX 1

ALGAE

Algae represent a low-input, high-yield feedstock to produce biofuels. Ethanol can be obtained principally through fermentation; microalgae have the ability to produce H_2 under Sulphur deprivation; under Nitrogen deprivation, they can increase their lipid content (up to 50% dry biomass) from which biodiesel can be processed.

According to many lab studies, compared to traditional crops, the advantages of deriving biofuel from algae include rapid growth rates (they can grow 100 times faster than plants and double their mass in one day- Murphy 2004) and a much higher per-unit of land yield (Chisti 2007). It is also a non-food crop and so does not compete with agriculture food crop for land; in addition, algae biofuel contains no sulphur, is non-toxic, and is highly biodegradable.

Microalgae have a flexible metabolism, which offers the possibility to modify their biochemical pathways and compositions.

Microalgae can be cultivated in high energy consumption closed systems (photobioreactors) or in open ponds (outdoor cultures) which have a better net energy ratio.

The main obstacles for large scale competitive microalgae biofuel cultivations are, at present, low photosynthetic efficiency and productivity (Tredici 2009).

Algae can, in fact, photosynthesise better at low irradiances (the antenna pigment systems are optimized for daily average intensity) and can rapidly achieve photo saturation; increased irradiance may also start a photo inhibition process that can lead to damage. Light saturation, furthermore, reduces productivity. In dense cultures algae adapt phenotypically to low light assembling pigment (acclimatization). In addition algae's rapid growth rate does not always coincide with an increase in biomass.

At present there are 30,000 known species of microalgae with perhaps hundreds of thousands more still to be identified.

Many species of algae cultivated in bioreactors cannot grow in open ponds due to contamination and competition with other algae species. From 1978 to 1996, the U.S. Department of Energy funded a programme to develop renewable transportation fuels from algae. The results of the study showed the difficulty of maintaining selected algae strains for long periods (> 1 year) in open ponds (Sheehan et al. 1998).

In recent years, however, tremendous advances have been made in the science of manipulating the metabolism of algae and the engineering of microalgae production systems (Tredici et al. 2009). Mutants production, which is also facilitated by a rapid growth rate, can produce new stable strains that may overcome the light-saturation effect, while improving solar energy conversion (i.e. by genetically truncating the size of the light-harvesting chlorophyll - Mitra et al. 2008); they also have a greater photosynthetic efficiency, thus enabling bigger biomass yield; finally they can survive for long periods in open ponds.

Worldwide commercialization for biofuel of alien, wild algae or of specific strains obtained through selection or genetic engineering from wild types can pose a threat in case of contamination of natural habitats.

Escapes from open ponds (through normal dispersion agents), photobioreactors (i.e. "backyard" bioreactors), or through strain exchanges/commercialization may generate irreversible damage to water ecosystems.

In Europe, damage to natural marine ecosystems and to human health caused from invasive microalgae (introduced from ballast water, aquaculture, or fouling) has already been documented (Wyatt et al. 2002; Maso et al. 2006). Proliferations of microalgae in marine or brackish waters can cause massive deaths among fish, alter ecosystems and poison humans eating seafood, and so damage also the tourism industry (HAB: 'Harmful Algal Bloom').

Transgenic algae should be grown in complete containment (Mayfield et al. 2007), or in “biogeographical” isolation, to reduce any risk of environmental contamination. Future commercialization or industrial development of strains which have been selected or engineered from wild types with traits that can increase photosynthetic efficiency and biomass productivity and may be able to survive in open ponds should be treated as pests, according to the International Standard for Phytosanitary Measures.

ANNEX 2

NATIVE SPECIES (cardoon)

Cardoon (*Cynara cardunculus* var. *altilis* –DC) recruits mainly from seeds and was domesticated from the wild artichoke (Rottemberg et al. 2005). *Cynara cardunculus* var. *sylvestris* is a perennial species with many local genotypes, which remain completely interfertile with cardoon (Raccuia et al. 2004; Sonnante et al. 2007).

According to Wiklund (1992) the species var. *sylvestris* can be divided into two different taxa: ssp. *cardunculus* (occurring in Italy - photo- on the eastern side of the Mediterranean basin) and ssp. *flavescens* (occurring in Sicily, in Spain and as a weed in other mediterranean-type climates such as Australia and California).

Cardoon, as a consequence of being domesticated from the “genetic pool” of a native species is adapted to mediterranean environments. The physiological and reproductive traits of cardoon make it a potential invasive species which could harm different types of habitats: Mediterranean, arid and semi-arid, grassland habitats of native thistle plant communities (*Onopordetea acanthii*; *Carthametalia lanati*), Natura 2000 habitats of pseudo-steppe with grasses and annuals (*Thero-Brachypodietea*) and in old fields (*Brometalia rubenti-tectorum*) where they could slow down the secondary succession re-naturalization processes.

Cardoon was initially domesticated for its stalk size and then selected for biofuel cultivations, especially from Spanish genotypes. Cardoon biofuel crop is distinguished by its rapid growth, efficient use of water resources and great reproduction capacity (Pignatelli et al 2006).

Surveys undertaken in Southern Italy mediterranean habitats within experimental farmland fields showed that cardoon “crop escape” was already underway (Crosti et al. 2008).

Consequently the taxon poses a double threat: it may lead to hybridization with different population of wild artichokes, and it may compete for natural resources with other native species especially in disturbed habitats. Species selected traits, together with the cropping system (annual planting, large scale intensive cultivations in different areas, harvest at senescence when pollination and seed dispersion likely already occurred) make it a potential invasive species even though domestication generally leads to reduced fertility.

Cardoon is already considered as a pest species in other mediterranean-type climate regions such as California, Western Australia and South Africa (Marushia et al. 2008). In particular the Spanish genotype seems to be much more aggressive than the Italian one (Holt & Garcia 2009). For these reasons, when the species is selected and cultivated as a biofuel crop, specific cultivation criteria are needed to limit the weedy behaviour (i.e.: use of non aggressive cultivars, cutting of flower heads, and establishment of a buffer zone).



Photo wild artichoke (left) and biofuel cardoon (photos taken the same day in the same area)



Photo Biofuel cardoon escaped from field



Prototype machine to harvest cynara capitula (photo CRA-ISMA)

ANNEX 3

WILLOWS AND POPLAR

Willows and Poplars belong to the same salicaceae family and are found primarily in moist soils along riverbanks and water courses. They are native to European temperate regions. Both are dioecious trees but bisexuality may occur especially in hybrids.

The physiological and ecological amplitude, along with their adaptation to a wide range of climates, make them suitable for cultivation. Both genera are becoming increasingly more important in biofuel plantation research, commercial enterprises and consequent hybridization studies (Venendaal et al. 1997).

Over the last decades, improvements in yields and tolerances to biotic and abiotic stresses have been achieved through genetic improvement. A considerable number of hybrids that offer higher yields, increased disease- or pest-resistance and fast growth have been produced (Kremer 2003; Kuzovkina 2008).

Both genera are capable of reproducing both sexually and vegetatively.

Recruitment from seed is restricted to bare soil kept moist for a long duration, from the time of seed set (in spring). Seeds have an extremely short period of viability when dry (less than 10 weeks with clear differences among the species) and germinate in about one day when imbibed. Seedlings need a lot of light, have slow root growth and suffer competition from other vegetation. Probably the main barrier to the survival of seedlings is the lack of sites suitable for germination and survival (safe sites), due to rising or rapidly falling soil moisture. Any flooding or drought during the first stages of recruitment will cause perishment. Stream banks in Europe are usually densely vegetated or densely settled and/or farmed. Consequently there are few safe sites for yearly recruitment.

In willow or poplar cultivations, recruitment usually does not occur due to the lack of a suitable site or regular soil clearing; seedlings may be more commonly found in nearby ditches or river channels.

The trees can easily grow from broken branches taking root in wet areas and this increases spreading, not only by nature but also through people taking cuttings.

Willows and poplars are very cross-fertile, and numerous hybrids occur; in addition, both artificial and natural hybrids have been produced for use in forestry and horticulture and planted throughout Europe. There is therefore concern about native trees losing their identity by breeding with the introduced taxa, especially with the increase of land destined for large scale biofuel plantations.

In their native habitats, the separate “identities” of species tend to be preserved by the existence of natural barriers for interbreeding (non-overlapping flowering times, non-overlapping geographical and ecological distributions) as well as genetic barriers (Mosseler and Papadopol 1989). Some of the barriers to hybridisation tend to break down when trees are introduced to new environments, where flowering times may be altered or overlap. Hybridisation is more likely to occur where native species are planted side by side with introduced clones or cultivar.

Offspring of planted hybrids are able to invade sites where the native trees regenerates, and the genome of the native species can be invaded by alien genes (White 1994; Heinze 2008).

The flow of genetic material between species, populations, clones and cultivars may contribute to the loss of native germplasm. Introgression of new genetic material can lead to the replacement of local genotypes that can be threatened with extinction when new alleles or genes are introduced.

If in the future, larger areas are managed for cultivation we might expect some taxa to have more invasive characteristics (Lefèvre et al. 2001) also as a consequence of a new biofuel cropping system.

To reduce the spread of alleles and recruitment of introduced taxa, the following cultivation criteria should be applied:

Use of sterile cultivars; planting cuttings only from purely male/female trees; distance between cultivation of different sex; use of less aggressive cultivar to reduce cross-pollination and

seed/vegetative propagation; and by setting a minimum distance between the tree plantation and native populations (to avoid interbreeding) and water streams (to avoid the spread of pieces of broken branches that may float down river and take root in available sites).

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