DEvelopment of a quick Monitoring index as a tool to assess Environmental impacts of TRAnsgenic crops (DEMETRA)

9 788890 365386

www.life-demetra.eu

Beneficiary's name

Plant Genetics Institute, UOS Firenze, CNR (Coordinator)
Dipartimento di Gestione dei Sistemi Agrari, Alimentari
e Forestali (GESAAF),
University of Florence
Ente Parco Regionale Migliarino San Rossore Massaciuccoli
Regione Toscana

Project duration

01/01/2010 - 30/06/2013

Project's contact details
Coordinator and scientific responsible of the project

Cristina Vettori - CNR-IGV
Madonna del Piano 10
50019 Sesto Fiorentino
Firenze (Italia)
Uff. +39 055 5225728
Fax +39 055 5225729
cristina.vettori@cnr.it

Project Manager

Laura Bartalucci - Regione Toscana
Via di Novoli, 26
50127 - Firenze (Italia)
Uff. +39 055 4385268
Fax +39 055 4383134
laura.bartalucci@regione.toscana.it

Technical coordination

Communication Manager
Alvaro Fruttuosi - Regione Toscana

Communication staff

Marco Sulas - Regione Toscana
Simonetta Demuro - Regione Toscana

Website

www.life-demetra.eu

DEvelopment of a quick Monitoring index as a tool to assess Environmental impacts of TRAnsgenic crops (DEMETRA)

Regione Toscana

Ente Parco Regionale Migliarino San Rossore Massaciuccoli

UNIVERSITÀ DEGLI STUDI FIRENZE
GESAAF
DIPARTIMENTO DI GESTIONE DEI SISTEMI AGRARI, ALIMENTARI E FORESTALI

DEvelopment of a quick Monitoring index as a tool to assess Environmental impacts of TRAnsgenic crops (DEMETRA)

Chapter 1

Description of the Studied Areas

Cristina Vettori[1], Francesca Bottalico[2], Lorenzo Chelazzi[3], Silvia Fiorentini[2], Donatella Paffetti[4], Valeria Tomaselli[5], Davide Travaglini[2]

[1]Plant Genetics Institute, Division of Florence, National Research Council, Via Madonna del Piano 10, 50019 Sesto Fiorentino (FI), (Italy); [2]Department of Agricultural, Food and Forestry Systems, University of Florence, Via San Bonaventura 13, 50145, Florence (Italy); [3]Institute of Ecosystem Study, Division of Florence, National Research Council, Via Madonna del Piano 10, 50019 Sesto Fiorentino (FI), (Italy); [4]Department of Agriculture, Food and Environmental Sciences, University of Florence, P.le delle Cascine 28, 50144 Florence (Italy); [5]Plant Genetics Institute, National Research Council, via G.Amendola 165/A, 70126 Bari (Italy)

The studied areas are located in the Regional Park of Migliarino – San Rossore – Massaciuccoli, in the Pisa province (Tuscany, Italy) (Fig. 1). The protected area is characterized by a wide diversity of ecosystems and a high presence of lands suitable for agriculture. The project focused on three study areas characterized by different ecosystems.

Area 1 (Massaciuccoli Lake)

Fig. 1. Map of the project study area.
1. Wetland area of Massaciuccoli Lake;
2. Natural poplar forests and non cultivated areas;
3. Mixed and pine forests, wetlands, cropped area.

The "Lago e Padule di Massaciuccoli" (Cod. Natura2000 IT5120021) covers a total area of 1,908.01 ha, and it is a "Site of Community Importance" (SCI) according to the directive 92/43/CEE. Additionally, the SCI overlaps the "Site of Regional Importance" (SIR) according to the Regional Law L.R. 56/2000.

This site is entirely included in the Regional Park of Migliarino – San Rossore – Massaciuccoli and in the contiguous area. A protected area managed by LIPU (Italian League for Protection of Birds) is inside the site here considered.

The land of the project area is designed to agriculture and it falls in a private property.

Lake of fresh water surrounded by helophytic formations (reeds and *Cladium* spp.), bog and upland vegetation. The area is also characterized by upland forests, deep puddles of water belonging to sandy extraction. It is characterized by phytocenosis with *Cladium mariscus* and by other rare species, such as *Periploca graeca*

(one of the few Italian stations), and *Drosera rotundifolia* a very rare upland species in the bog.
Massaciuccoli Lake and its surrounding marsh area is one of the most important wetland habitats in Italy. It is characterized by a large lake slightly deep, on average less than two meters, with obvious problems of eutrophization and wide marsh areas, particularly in northern part of the site.
The habitats of community interest (Annex I Dir. 92/43) are small depressions on peat substrate with a community of Rhynchospora alba and/or *R. fusca* (code 71.50) and the neutral-basophils peat bogs with a dominance of *Cladium mariscus* and/or *Carex davalliana* (code 72.10). Among the flora species *Marsilea quadrifolia* is included in the Annex II Dir 92/43.
The animal species are much more numerous; it is a very important site for migratory and wintering species of birds. Among the breeding species of greatest importance is certainly the bittern (*Botaurus stellaris*), for which the lake is the most important nesting area at national level; then, we underline the presence of *Ixobrychus minutus, Ardea purpurea, Himantopus himantopus, Circus aeroginosus and Acrocephalus melanopogon*. All these species are related mainly to the marsh areas and in particular reeds.
The amphibian *Triturus carnifex*, an Italian endemism, and the reptile *Emys orbicularis* are species of Community interest, cited in Annex II of Dir. 92/43/CEE.

Area 2 (Serchio River) and Area 3 (Arno River)

These study areas are in the SCI/SIR/SPA “Selva Pisana” (Cod. Natura2000 IT5170002) with a total area of 9,658.34 ha. This site is entirely included in the Regional Park of Migliarino – San Rossore – Massaciuccoli.
The land of the project area falls in the public property of the Tuscany Region and it is managed by the Regional Park of Migliarino – San Rossore – Massaciuccoli.
Sandy coast, mostly in regression, is characterized by the typical sequence of plant formations of sand heaths (*Cakile maritima* community, seseleto, *Elymus farctus* community, *Ammophyila littoralis* community, *Phragmites australis* community and gen. *Juniperus* community). The area is composed by both coastal dune habitats and internal system of fossil dunes and interdunes with alternance of pine forests with *Pinus pinaster* or *Pinus pinea*, high bush, freshwater and brackish wetlands, wide forests with *Quercus robur*. The internal wetlands are characterized by mosaic of *Salicornia* sp.pl. community, plant formations of helophytes, such as *Phragmites australis* community e *Carex* sp.pl. community, and former farmland flooded in winter.
Dunal ecosystems and humid retrodunal areas host habitats and species of flora and fauna with high conservatory interest.
One of the 2-3 best examples of dunal habitat in Tuscany, slightly influenced by man, is included in the considered area. Dunal habitat of this area represents one of the best ones along the Tyrrhenian coast and, together with the neighbouring site “Coastal Dune of Torre

del Lago", the only one in a good state of conservation in the northern Tuscany.
These considerations are related to habitats of UE interest, such as "Embryonic shifting dunes", "Shifting dunes along the shoreline with *Ammophila arenaria* (white dune)", "Annual vegetation of the lines of marine deposit", and to priority habitats, such as "Coastal Dunes with *Juniperus* spp." and, in back-dune area, "*Calcareous fens* with *Cladium mariscus* and *Caricion davallianae* spp.".
The wetlands, included in the area here considered, are interesting at National and sometimes at International level, for the wintering of waterfowl and for the rest during migration along the Tyrrhenian coast route.
Dunal and retrodunal habitat host rare plant species, such as *Solidago virgaurea* spp. *litoralis* (an endemism of versiliese-pisano sandy coast), *Stachys recta* var. *psammofila* (endemism of the Tyrrhenian coast), *Periploca graeca* (one of the few Italian stations), and several nesting populations of UE interest species (in particular *Burhinus oedicnemus*). Reproductive populations of *Rhinolophus ferrumequinum* and *Myotis emarginatus*, species included in the Annex II of Dir. 92/43/CEE, live in the considered area. *Rhinolophus ferrumequinum*'s colony is the only one known in Tuscany and the biggest in Italy; some individuals of this colony winter in a building inside the site.

In each area, permanent study areas and, within them, the sub-areas (transect, plot, etc.) needed for data collection have been identified (Table 1).

Table 1. The study areas.

	Locality	System – sub-area	Sampling sites
AREA 1 (Massaciuccoli Lake)		Lake	6 points for biodiversity 32 poplar
		Poplar plantation	30 poplar
AREA 2 (Serchio river)	Ontanelli	Not cropped area-parcel 821	21 sub-plots
	Fortino nuovo	Natural poplar forests	21 sub-plots 30 poplar
	Fortino nuovo	Poplar plantations	30 poplar
AREA 3 (Arno river)	Culatta	Cropped area-Parcel A6	Crops
	Culatta	Mixed forest	43 sub plots, 267 *loro campestre*
	Colmate del Bozzone	Pine forest	7 sub-plots
	Colmate del Bozzone-lame	Wetland area	10 plots

Vegetational description of the sampling site of the permanent study areas

During the first months of operation plot/sampling points, some plants and trees (poplar and maple) of interest for the project have been defined. The plots were staked, the plants marked with labels, and the geographic coordinates of the plots were determined using a GPS and registered in a GIS. The plots of study were defined according to the scheme of Dengler (2009) using a variable number of modules based on the extension of the area under examination. In particular, each module has a squared area of 1,000 m^2 with smaller areas of 100 m^2 and 10 m^2 along the main diagonal of the square. Within these

squares investigations of plant biodiversity, animal and microbial have been carried out (Chapter 4). The identification of permanent plots allowed to repeat the investigations of biodiversity in the same points for the duration of the project allowing to compare data between different years.

Study Area 1 (Massaciuccoli Lake)

Massaciuccoli Lake has an extension of 2,000 hectares and is the most important wetland area in Tuscany. The particular climate of this area has allowed the survival of relict vegetation. The bird population is very diversified. The lake is an important site for nesting and resting place for migratory species: in fact there are over 260 birds species. The study area, called "Anghetto", is approximately 30 ha and is located near Massaciuccoli in the municipality of Massarosa. It is bounded NW from Massaciuccoli Lake and the rest by canals (ditch Navicello) communicating with the lake itself. The vegetation is mainly made up of *Phragmites australis* (reed) in parts of the border, with the lake and the canals, and *Cladium mariscus* (sedge) in the central parts.

Fig. 2. Study Area 1 (Massaciuccoli Lake).

The whole area is almost always flooded except the outer edges, embankments, and some points raised where some examples of spontaneous poplars are also present.

In addition to the straw and the sedge, the vegetation of this environment is characterized by the presence of *Solanum dulcamara, Iris pseudoacorus, Osmunda regalis, Hibiscus palustris and Periploca graeca*.

In the waters of the canals and of the lake, *Ceratophyllum demersum, Potamogeton pectinatus, Utricularia australis, Lemna gibba and Myriophyllum spicatum* are common species.

Because of the particular type of the site, "points" for sampling, distributed on the area south of the lake, have been identified and in which the investigation of plant (grasses) and animals biodiversity were made. In addition, the poplar individuals were identified to study the genetic diversity and the gene flow (breeding) with a poplar plantation present near the southwest edge of the lake (Fig. 2).

Study Area 2 (Serchio river)

- Natural poplar forest (Fortino nuovo Locality)

The area includes a mixed forest characterized by the presence of White poplar (*Populus alba* L.), Elm (*Ulmus minor* L.), Narrow-leafed Ash (*Fraxinus oxycarpa* Bieb.), Black alder (*Alnus glutinosa* L.), Grey poplar (*Populus x canescens* ((Aiton) Sm.)) and some individuals of peduncolate oak (*Quercus robur* L.). This training is similar to an irregular high forest

in which both individuals from seed or individuals developed from the stump are present. The coverage is uneven and regeneration is not present.
This area has a flat morphology, without rockiness outcropping. It is also easily accessible. It is approximately 7,400 m^2 large in which 3 plots have been identified. Moreover, a total of 30 poplar have been identified and the sex phenologically determined. Among these 6 female trees have been identified (Fig. 3).
An area of 2,500 m^2 was delimited in order to determine the qualitative and quantitative characteristics of the forest stand.
- Not cropped area - parcel S21 (Ontanelli Locality)
This is an area in which cultivation is not carried out since 10 years. This sub-area has a surface of approximately 14,000 m^2 in which 3 plots have been identified (Fig. 4).

Fig. 3. Study Area 2 (Serchio river): natural poplar forest (Fortino nuovo Locality).

Fig. 4. Study Area 2 (Serchio river): not cropped area – Parcel S21 (Ontanelli Locality).

Study Area 3 (Arno river)

- Mixed broadleaved forest (Culatta Locality)

The study area is located on a flat terrain, without rockiness outcropping. Furthermore, being situated on the edge of a forest road it is easily accessible.
The study area borders a mixed broadleaved forest characterized by the presence of Peduncolate oak (*Quercus robur* L.), Narrow-leafed Ash (*Fraxinus oxycarpa* Bieb.), Gray poplar (*Populus* x *canescens* ((Aiton) Sm.)), maple (*Acer campestre* L.), Common hornbeam (*Carpinus betulus* L.), elm (*Ulmus minor* L.), Black alder (*Alnus glutinosa* L.) and White poplar (*Populus alba* L.).
Forest cover is multi strata with oak trees of considerable size and younger individuals of other species with widely varying dimensions. The stand density is not uniform. Crown cover is uneven with openings due to uprooted plants. The undergrowth is characterized by the significant presence of elmleaf blackberry (*Rubus ulmifolius* Schott) and common hawthorn (*Crataegus monogyna* Jacq.).
The regeneration is limited probably due to the excessive load fauna that persists over the area. This formation lies in the association of *Fraxino angustifoliae-Quercetum roboris* Gellini, Pedrotti, Venanzoni 1986.
In this sub-area of about 118,000 m^2, 6 plots were selected and two further plots of only 100 m^2 were identified near the edge of the forest. In addition, a total of 267 individuals

of *Acer campestre* (open pollinated mainly entomophilous) were detected. Of these, 32 were then chosen so as to cover up significantly the distribution area to conduct genetic variability study (Fig. 5). An area of 2,500 m^2 was carried out in order to determine the qualitative and quantitative characteristics of the forest area (Fig. 5).

Fig. 5. Study Area 3 (Arno river): mixed broadleaved forest (Culatta Locality).

- Pine forest and wetland area (Colmate del Bozzone Locality – Lame)

In this area two types of vegetation formation were considered.

The first type is a pure Italian stone pine forest (*Pinus pinea*) 100-120 years old. Pine trees have large crown which lead to an almost complete ground cover. The undergrowth is mainly composed by the herbaceous layer of grasses.

The second type of vegetation is an open area characterized by a large grassy area that most likely comes from reclaimed marshlands.

These two previous situations are divided by a thick bed of reeds.

In this sub-area of about 25,000 m^2, one plot has been identified in the pine forest and an area of 2,500 m^2 was carried out in order to determine the qualitative and quantitative characteristics of the forest stand.

Fig. 6. Study Area 3 (Arno river): Pine forest and wetland area (Colmate del Bozzone Locality – Lame).

Additionally 10 plots each of 100 m^2 have been identified in the wetland area adjacent to the pine forest (Fig. 6).

- Cropped area - parcel A6 (Culatta Locality):

This area is located within an area used for agricultural from MSRM Regional Park, where it was not possible to identify the permanent study plots as not to hinder the activities of the Park. In this area, maize, sunflower and oil-seed rape were cropped for the study (Fig. 7). In particular, the investigations pollen flow and breeding between the oil seed-rape cultivated and wild relative were conducted.

Fig. 7. Study Area 3 (Arno river): cropped area - parcel A6 (Culatta Locality) in which maize, sunflower and oil-seed rape were cropped. cultivation in 2012 year.

Characterization of forest structure

In order to characterize the species composition and the structure of forest formations in the Park of MSRM, forest structure was investigated in the following study areas: “Natural poplar forest” (Area 2), “Mixed broadleaved forest” (Area 3), “Pine forest and wetland area” (Area 3). In each sub area, field works were carried out within a squared plot 2500 m^2 large, and the positioning of living trees, dead trees (standing dead trees and lying dead trees), and dead wood on the ground was determined.

For living trees, the species was noted and the following parameters were measured: the diameter at breast height (DBH), the total tree height, the height of base crown, and the projection of the crown in 4 directions (N, E, S, O). For dead trees the species was noted and the following parameters were measured: the DBH, the total tree height, and the decay class. For pieces of dead wood on the ground and for stumps, the species was noted and the following parameters were measured: diameter at the two ends, the total length (the height in case of stumps), and the decay class.

The characterization of flora and fauna in the forest ecosystems was supplemented with an analysis of the specific composition and spatial structure of forest stands in order to have a set of useful data for the development of indicators for monitoring and for their implementation map-based systems with GIS.

Table 2 shows the dendrometric parameters for “Natural poplar forest” (Study Area 2 Serchio river, Fortino nuovo Locality). The stem number-diameter distribution in diameter classes of 5 cm is shown in Figure 8.

Table 2. Natural poplar forest: number of trees, basal area and volume per hectare.

Species	Number n/ha	Basal area m^2/ha	Volume[1] m^3/ha
Maple	4	0.1	0.4
Narrow-leafed Ash	44	8.0	138.3
Elm	28	1.4	10.1
Black alder	188	8.0	77.7
Poplar	20	8.0	141.7
Total	**284**	**25.4**	**368.2**

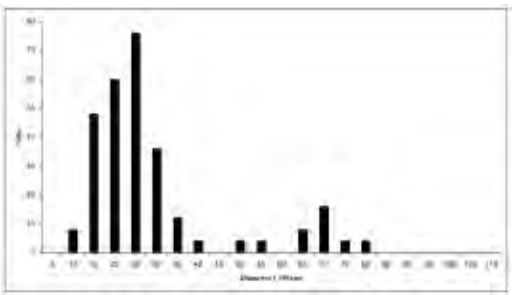

Fig. 8. Stem number-diameter distribution for “Natural poplar forest”.

Table 3 shows the dendrometric parameters for “Mixed broadleaved forest” (Study Area 3 Arno river). The stem number-diameter distribution in diameter classes of 5 cm is shown in Figure 9.

Table 3. *Mixed forest: number of plants, basimetric area and volume per hectare.*

Species	Numer n/ha	Basal area m^2/ha	Volume^2 m^3/ha
Maple	44	1.3	11.3

Species	Numer n/ha	Basal area m^2/ha	Volume^2 m^3/ha
Whitethorn	20	0.3	0.6
Peduncolate oak	28	9.5	151.5
Narrow-leafed Ash	436	15.4	192.5
Elm	80	1.6	11.7
Total	**608**	**27.9**	**367.5**

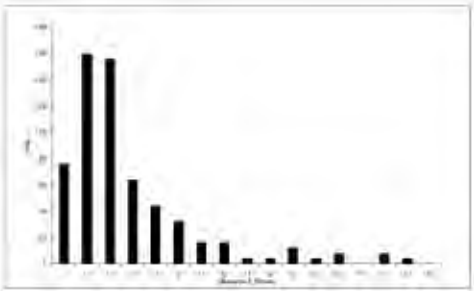

Fig. 9. Stem number-diameter distribution for “Mixed broadleaved forest”.

Table 4 shows the dendrometric parameters for Italian stone pine forest. The distribution of the number of trees in diameter classes of 5 cm is shown in Figure 10.

Table 4. *Pine forest: number of trees, basimetric area and volume per hectare.*

Species	Number n/ha	Basal area m^2/ha	Volume^2 m^3/ha
Pine	92	33.2	517.9
Total	92	33.2	517.9

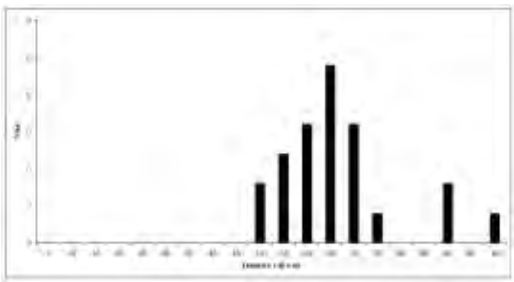

Fig. 10. Stem number-diameter distribution.

Crops done from 2010 to 2012

The crops of maize, sunflower and oilseed rape made and used during the project are summarized in the following table:

Locality	Crops in 2010	Crops in 2011	Crops in 2012
1. Massaciuccoli Lake			Private poplar plantation
2. Ontanelli, plot S23		Poplar plantation presents in MSRM	
3. Fortino Nuovo		Poplar plantation presents in MSRM	

Locality	Crops in 2010	Crops in 2011	Crops in 2012
4. Culatta, plot A6	Maize; Sunflower	Maize; Sunflower; Oil seed-rape*	Maize; Sunflower;
5. Culatta, plot A9		Maize	
6. Migliarino (close to A11)	Oil seed-rape* (cropped by the private Marchese Mazzorosa)		

* The oil seed-rape is autumnal cultivar, therefore it is seeded in August/September and the pollen production occurs in the spring (March/April) the following year.

Types of weather stations

In consideration of the needs of the project, the weather stations have different characteristics according to the study area in which they are located (Fig. 11):

1. Weather station 1 (Area 1 - Massaciuccoli Lake) measures the following parameters:
- Intensity and wind direction at a height of 4 m above the lake;
- Temperature and humidity of the air at a height of 3.5 m above the lake;
- Rain.

2. Weather Station 2 (Area 2, Ontanelli locality) measures the following parameters:
- Intensity and wind direction in three installments (2.5 m, 5 m, and 10 m);
- Air temperature and humidity at 2 m;
- Temperature and soil moisture at a depth of 10 cm;
- Rain.

3. Weather Station 3 (Area 3, Culatta locality) measures the following parameters:
- Intensity and wind direction in three installments (2.5 m, 5 m, and 10 m);
- Air temperature and humidity at 2 m;
- Temperature and soil moisture at a depth of 10 cm;
- Rain.
- Atmospheric pressure
- Solar radiation (global, diffuse, sunshine, UVA, UVB)

[1] The volume was computed using double entry volume equations (I.F.N.I., 1985).
[2] The volume was computed using double entry volume equations (I.F.N.I., 1985)
[3] The volume was computed using double entry volume equations (I.F.N.I., 1985)

Every 15 minutes the average, or the sum for rain and sunshine duration is performed and stored.
The power supply of tools and acquisition systems is provided by photovoltaic panels with buffer batteries.

Fig. 11. Images of the weather stations in the 3 study areas.