Are Teosinte and Feral Maize present in the Netherlands?

Ing. H.F. Huiting, dr.ir. M.M. Riemens & dr.ir. R.Y van der Weide

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Contents

Preface

The Netherlands Commission on Genetic Modification (COGEM) frequently evaluates whether genetically modified maize could pose a threat to the environment. In such an evaluation, it is essential to have accurate information on the presence of populations of maize and its crop wild relatives in the country. Until recently, it was common belief that such populations were not present in the Netherlands. However, recent reports of feral maize plants and of the maize wild relative teosinte in several countries of Europe gave rise to the concern that populations of feral maize and teosinte might be spreading and could already have reached (or would ultimately reach) the Netherlands.

Therefore, the COGEM commissioned research with three objectives:

1. to obtain up-to-date information on spatial distribution of the occurrence of maize plants outside arable land in the Netherlands and on the source of these plants.
2. to obtain up-to-date information on the temporal distribution of the occurrence of maize plants outside arable land in the Netherlands, i.e. to obtain insight into the periods of the year when such maize plants occur and whether there are any trends visible in the duration of these periods.
3. to determine whether teosinte species are currently present in the Netherlands and, if so, to describe in detail any teosintes identified.

This research assignment to some extent follows up on earlier research commissioned by the COGEM in 2011, entitled: "Crop volunteers and climate change: Effects of future climate change on the occurrence of maize, sugar beet and potato volunteers in the Netherlands" (COGEM Research Report CGM 2011-11).

During several sessions with the researchers, the Advisory Committee evaluated progress and analyses of the research and provided suggestions on methodologies and reporting. These meetings were always interesting, not the least because of the complexity of the taxonomy of maize and its wild relatives and the confusion in literature on definitions of volunteers, feral plants and self-sustaining populations. The Advisory Committee also supported the writing process by commenting on several drafts.

The Advisory Committee is convinced that this report provides an accurate account of the current situation of the occurrence of feral maize and teosintes in the Netherlands, based on a diligent search for information from a wide diversity of sources.

Prof. dr P.C. Struik,
Chair Advisory Committee

Advisory Committee
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Dr. T.J. de Jong, Leiden University, Institute of Biology Leiden
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Summary

As part of the European market approval process, the Netherlands Commission on Genetic Modification (COGEM) assesses whether a genetically modified (GM) maize breeding line could pose an environmental hazard if imported or cultivated in the Netherlands. When assessing the risks GM maize poses to the environment, insights into the presence of maize and its interbreeding with wild variants are important. Based on the reported presence of 'feral' maize plants in Austria and of its relative teosinte in France and Spain, COGEM initiated investigations to determine whether feral maize and teosinte now occur in the Netherlands. This report provides up-to-date background information for use in assessment of possible GM maize risks.

The research comprised two main steps: description of teosinte (sub)species, maize and other relevant monocotyledon species and definition of self-sustaining, feral and volunteer and determination of the presence of feral maize and teosinte in the Netherlands. In determination of whether teosinte and feral maize occur in the Netherlands, a combination of different strategies was applied: 1) consultation of databases; 2) tracking of retailed teosinte seeds; and 3) network consultations with maize breeders, maize cultivation experts and pesticide suppliers.

Although teosinte seeds are sold commercially in the Netherlands, we could not find any distribution in nature or environment yet. The network consultation and distribution of information and the call to report teosinte yielded no reports of teosinte in maize cultivation areas, although the vast majority of maize experts and advisors in the Netherlands were reached and we did receive reports on lookalikes.

It is widely claimed that maize as a species cannot form a self-sustaining population under (semi-) natural conditions, even not in southern Europe with mild winter conditions. The feral maize reported in Austria seems to be from seed spill or volunteers, with no indications of self-sustaining populations. Based on the data in this report, there is no indication that the current situation in the Netherlands is different, as there have been no reports at all of possible self-sustaining maize populations. This makes the risk of development of self-sustaining populations in the Netherlands in the near future very unlikely.

Even feral maize populations and volunteers are scarce and this situation is not expected to increase much, even considering climate change in the Netherlands. Because maize seeds lack dormancy they germinate before winter and die before the next season. However, the current perception among farmers and advisors regarding maize volunteers is that numbers in following crops are scarce but may be rising. Recommendations towards better and more detailed insight in the volunteer and/or feral status of maize are 1) to ask farmers and advisors to record (qualitative or indicative quantitative data) volunteers; 2) to implement a scientific monitoring programme (to obtain solid quantitative data); and 3) to instruct/invite Dutch National Database Flora and Fauna biologists to make observations of maize volunteers in fields (quantitative and qualitative data). Recommendations to monitor occurrence of teosinte are 1) to follow up on seed sold; 2) to monitor the weed situation in farmers' fields by enhancing awareness of new weed species; and 3) to interview weed control experts regularly.

Samenvatting

Als onderdeel van de het Europese registratieproces, beoordeelt de COGEM of genetisch gemodificeerd (GG) kweekmateriaal van maïs een milieukundige bedreiging zou kunnen vormen als dit wordt geïmporteerd of geteeld in Nederland. Bij deze beoordeling van risico's van GG maïs is inzicht nodig in de aanwezigheid en uitkruising van mais en wilde varianten. Naar aanleiding van in Oostenrijk gerapporteerde 'verwilderde' mais en de aanwezigheid van teosinte – de wilde verwant van mais – in Spanje en Frankrijk heeft de COGEM onderzoek opgestart met als vraag of teosinte en verwilderde maïs voorkomen in Nederland. Dit rapport levert actuele achtergrondinformatie om te gebruiken bij beoordeling van mogelijke risico's van GG mais.

Het onderzoek omvat twee hoofdbestanddelen: een beschrijving van (onder)soorten teosinte, maïs en andere relevante monocotyle soorten inclusief een definitiebepaling van de mate van verwildering en het bepalen van de aanwezigheid van verwilderde maïs en teosinte in Nederland. Bij het bepalen of verwilderde maïs en teosinte voorkomen in Nederland is een gecombineerde strategie gebruikt: 1) raadplegen van databanken; 2) opsporen van verkocht teosintezaad en 3) raadplegen van een netwerk aan maisveredelaars, maisteeltexperts and leveranciers van gewasbeschermingsmiddelen. Hoewel teosintezaad wordt verkocht in Nederland, hebben we de soort niet gevonden in de natuur of op de akkers; raadplegen van het netwerk en de oproep om het voorkomen van teosinte te melden leverde geen meldingen van teosinte in maisteeltgebieden op, hoewel een grote meerderheid van experts en adviseurs op het gebied van maïsteelt bereikt werd. Er werden wel op teosinte lijkende planten gerapporteerd.

Alom wordt aangenomen dat maïs geen zichzelf in stand houdende populaties kan vormen in een (semi-)natuurlijke situatie, zelfs niet in het zachte winterklimaat in Zuid-Europa. De verwilderde maïs die is gerapporteerd in Oostenrijk lijkt te zijn voortgekomen uit morsen van zaad of kolven, zonder aanwijzingen van daadwerkelijk zichzelf in stand houdende populaties. Gebaseerd op de gegevens in dit rapport is er geen aanwijzing dat de huidige situatie in Nederland afwijkt, omdat er geen enkele melding is gemaakt van dergelijke populaties. Dit maakt dat de kans op zichzelf in stand houdende populaties in de nabije toekomst zeer onwaarschijnlijk.

Zelfs verwilderende maïspopulaties en opslagplanten zijn zeldzaam en een toename wordt vrijwel niet verwacht, zelfs met inachtneming van effecten van klimaatverandering in Nederland. Omdat maïszaad geen kiemrust kent zal gemorst zaad meest kiemen voor de winter en doodgaan voor het volgende seizoen. De indruk onder telers en adviseurs over opslagmaïs is niettemin dat aantallen opslagplanten weliswaar laag zijn, maar mogelijk stijgen. Aanbevelingen voor het verkrijgen van een beter en nauwkeuriger inzicht in de mate van verwildering van maïs zijn 1) om maïstelers te vragen om voorkomende opslagmaïs vast te leggen (kwantitatieve of kwalitatieve gegevens); 2) om een wetenschappelijk monitoringsprogramma op te zetten (om betrouwbare kwantitatieve gegevens te verkrijgen) en 3) om biologen actief voor de Nationale Databank Flora en Fauna te vragen maïsopslag in maispercelen waar te nemen (kwantitatieve of kwalitatieve gegevens). Aanbevelingen om teosinte te monitoren zijn 1) opvolgen van zaad dat wordt verkocht; 2) de onkruidsituatie in percelen opvolgen door alertheid voor nieuwe onkruidsoorten te verhogen en 3) door regelmatig onkruidbestrijdingsexperts te raadplegen.

1 Introduction

As part of the European market approval process, the Netherlands Commission on Genetic Modification (COGEM) assesses whether a genetically modified (GM) maize breeding line could pose an environmental hazard if imported or cultivated in the Netherlands. When assessing the risks posed by GM maize to the environment, insights into the presence of maize and its interbreeding with wild variants are very important. Maize volunteer plants are known to be present in the Netherlands, but these are assumed to grow from spilled seed. Maize is assumed to be unable to establish in the wild here, but this has not been verified in recent years. Maize is able to interbreed to different extents with teosinte, a group of eight wild grasses in the Poaceae family that includes other subspecies *Zea mays*. Teosinte occurs as a weed in Spain and France (EFSA, 2016) and may be emerging elsewhere. As a result of a recent publication reporting the presence of 'feral' maize plants in Austria, COGEM initiated investigations into the situation in the Netherlands, to determine whether feral maize occurs and whether teosinte has been reported in the Netherlands. Presence of teosinte species able to interbreed with maize would affect the current risk assessment of GM maize cultivars, making early detection of teosinte important. Background information regarding feral maize and presence of teosinte in the Netherlands is an important component in assessing possible risks of GM maize.

1.1 Aim of the research

The aim of this research was to obtain up-to-date background information for use in assessment of possible GM maize risks. A primary objective for COGEM was to gain information on maize plants growing outside fields in the Netherlands and on the source of these plants or populations. Information on the season(s) in which maize occurred and on any possible changes in the length of this period was also considered important.
A second objective for COGEM was to determine whether teosinte species are now present in the Netherlands and to obtain detailed descriptions of any teosintes identified, based on the literature and on descriptions of plants growing from purchased seeds.

2 Materials & methods

2.1 Description of teosinte, maize and other relevant species

A description of teosinte species characteristics was made based on the existing literature and on observations made on growing maize and teosinte from seed. Together with these *Zea* species, a few other monocotyledon species were described, as these bear a high resemblance to *Zea* species during early stages of development in the field.
Teosinte seeds labelled as *Zea mexicana* were ordered from Vreeken's Zaden, a seed supplier in the Netherlands.

2.2 Occurrence of teosinte and feral maize in the Netherlands

In determination of whether teosinte and feral maize occur outside cultivated fields in the Netherlands, a combination of different strategies was applied. These comprised: 1) consultation of databases; 2) tracking of teosinte seeds supplied; and 3) network consultations with maize breeders, maize cultivation experts and pesticide suppliers.

2.2.1 Consultation of databases

The website www.waarneming.nl (hosted by Stichting Natuurinformatie) was checked for recent reports of maize and teosinte outside fields (search term *Zea*). Particularly for maize, reports were traced back to 2015 in order to establish whether these reports dealt with feral maize. This was done by visiting the sites in question in August 2018. The website www.verspreidingsatlas.nl (hosted by the Dutch National Database Flora and Fauna) was consulted for observations of maize outside fields, as well as for observations of several other plant species (search term plant species names), in order to put the data in perspective.

2.2.2 Tracking of supplied teosinte seeds

For around a decade, teosinte seeds have been sold in small quantities by Vreeken's Zaden, Dordrecht. In response to our queries, the company reported that these seeds were sold mainly to teaching gardens in the Netherlands, but it would not supply contact information about its customers. Therefore, over 50 teaching gardens in the Netherlands were asked whether they had ordered and planted teosinte in 2018.

2.2.3 Network consultation

In autumn 2017, a poster was produced, aiming at: 1) creating awareness within the agricultural community in the Netherlands of possible presence of teosinte in their or nearby fields and 2) urging this community to be on the lookout in their fields for unknown plants that might be teosinte. The poster (Appendix 1 in this report) was displayed, and flyers were handed out, at two forage crops demonstration days, on 5 and 14 September 2017, visited by hundreds of farmers and advisors. After these open days, the poster was also displayed in newsletters and websites of Ruwvoer en bodem (www.ruwvoerenbodem.nl), Koeien en kansen (www.koeienenkansen.nl), Wageningen University & Research and AgriHolland (www.agriholland.nl). In addition, it was shared through several accounts on LinkedIn (www.linkedin.com) and was physically displayed in the main entrance of the Wageningen University & Research Field Crops (WUR-FC) office, in Lelystad (with over 1,000 visitors yearly). Following up on publication of the poster, over 100 members of the agricultural advisory network were asked if teosinte had possibly been noted in or outside fields and if feral maize was present outside fields. This was done through two email consultation rounds, in February and September 2018. In the first round, the emphasis was on notification of field observations of unknown monocotyledon species that could possibly be teosinte. In the second round of emails, the findings during the season in 2018 and the overall impression of presence of feral maize were highlighted.

3 Results & discussion

3.1 Species description

Within the framework of the COGEM objectives, insights into the taxonomic classification of the genus *Zea* and similar (weed) species were sought in order to help understand and interpret the available data.

3.1.1 The genus *Zea*

The genus *Zea* from the Poaceae family is native to the Central American countries Mexico, Guatemala, Honduras and Nicaragua (Hubbard *et al*., 2012; Figure 1). Nowadays, it occupies a wide range of habitats, with diverse ecologies, ranging from semi-cold to warm thermal zones (Figure 2). The genus *Zea* includes maize and teosinte. Both perennial and annual teosinte species exist. The annual species are single-stalked, resembling maize, whereas plants of the perennial species also spread vegetative through rhizomes.

Taxonomic classification of the genus *Zea* has been the subject of debate and changes over the years. The classification made by Wilkes (1967) distinguished six teosinte races, based on geographical location and environmental differences. The classification described by Iltis & Doebley (1980) and Doebley & Iltis (1980) contained seven (sub)species and laid the basis for the classification system used today. In the current classification, the genus *Zea* is divided into two sections: *Zea* and *Luxuriantes*. *Zea mays* is the only species in the *Zea* section (with four subspecies), while the *Luxuriantes* section today consists of three to five species, without subspecies (Table 1). To illustrate the ongoing debate, Iltis & Benz (2000) describe *Z. nicaraguensis* as a separate species, but it may also be classified as *Z. luxurians*. Moreover, together with *Z. mays ssp. huehuetenangensis*, *Z. mays ssp. mexicana* and *Z. mays ssp. parviglumis*, *Z. luxurians* is classified as *Z. mexicana* in some publications. Gómez-Laurito (2013) describe yet another (8^{th}) teosinte species, *Zea vespertilio*, demonstrating that classification work is still in progress.

Table 1. *Classification of species and subspecies within the genus* Zea*, their ploidy and their country of origin. Based on Iltis & Doebley (1980), Doebley & Iltis (1980), Iltis & Benz (2000), Gómez-Laurito (2013) and others.*

Species	Subspecies	Name	Section	Ploidy	Origin
Zea diploperennis		Teosinte	Luxuriantes	n = x = 10	Mexico
Zea perennis		Teosinte	Luxuriantes	n = 2x = 20	Mexico
Zea luxurians		Teosinte	Luxuriantes	n = x = 10	Mexico, Guatemala, Honduras
Zea mays	*huehuetenangensis*	Teosinte	*Zea*	n = x = 10	Guatemala
	mexicana	Teosinte	*Zea*	n = x = 10	Mexico
	parviglumis	Teosinte	*Zea*	n = x = 10	Mexico
	mays	Maize	*Zea*	n = x = 10	Mexico
Zea nicaraguensis		Teosinte	Luxuriantes	n = x = 10	Nicaragua
Zea vespertilio		Teosinte	Luxuriantes	n = x = 10	Nicaragua

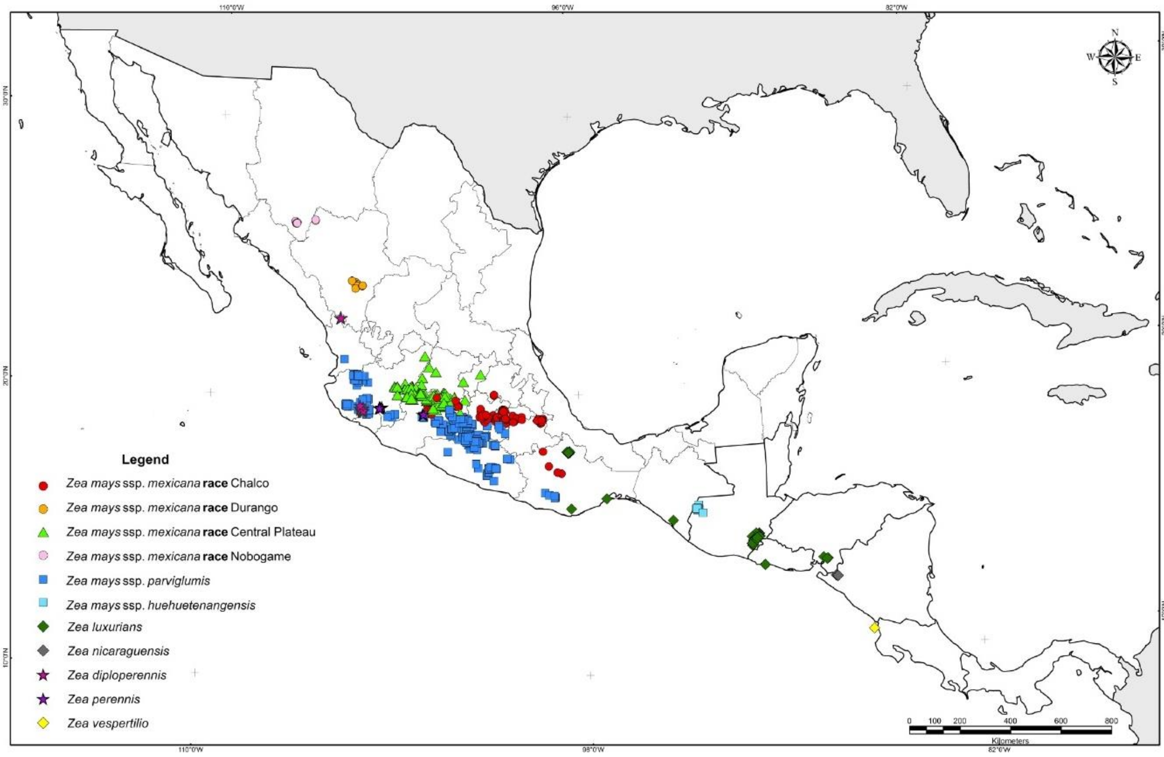

Figure 1. *Map showing the distribution of teosinte species, subspecies and four* Z. mays *subsp.* mexicana *races in Central America (González* et al.*, 2016).*

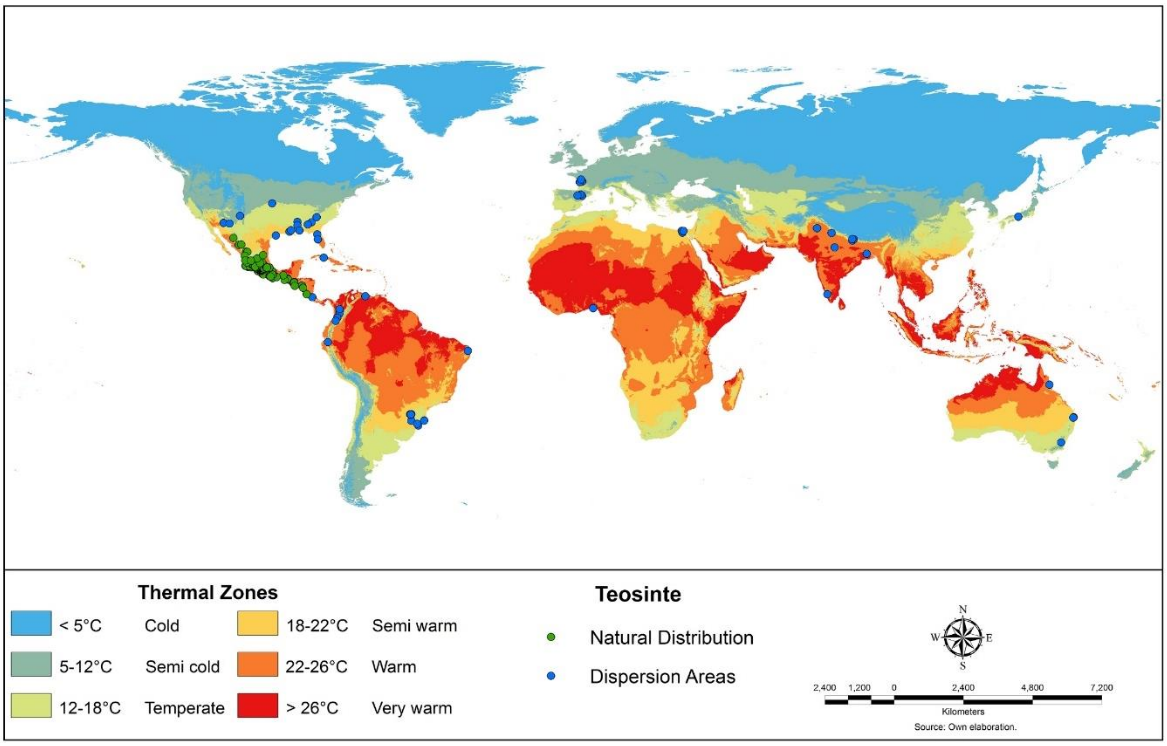

Figure 2. *Map showing the distribution of teosinte in different climate zones world-wide (González* et al.*, 2016).*

3.1.2 Differences between teosinte and maize

Maize (*Zea mays* subsp. *mays*) was domesticated from its wild relative teosinte about 9,000 years ago in southern Mexico. Domesticated maize, including high-yielding hybrid maize varieties grown in

Europe, is strictly monopodic, with non-shattering kernels that remain tightly attached to the cob, in contrast to the shattering kernels of the cobless, and highly branched, teosinte (Triketova *et al.*, 2017).

The key difference between maize and teosintes is the difference in apical dominance, which is strong in maize and weaker in teosintes (Hubbard *et al.,* 2002). This results in differences in the level of branching (virtually absent in maize and well present in teosintes) and the number of female inflorescences (1-2 in maize and at least one in each branch in teosintes). The spikes in teosinte mature to form a two-ranked ear, with 5-10 triangular or trapezoidal segments, whereas in maize the typical cob is formed (Figure 3). The male inflorescences (tassels) of teosinte and maize are considerably more similar, but male inflorescences in teosintes outnumber those in maize, as they appear at each apex. The axillary branches in maize are short and feminised, whereas the axillary branches of teosinte are long and end in a male inflorescence under normal growing conditions.

Based on the above parameters, distinction between maize and teosinte is readily possible based on branching as soon as generative development starts. However, should mutants occur, the situation changes. Previous quantitative trait locus (QTL) and other molecular analyses suggest that the *teosinte branched1* (*tb1*) gene of maize contributes to the architectural difference between maize and teosinte (Hubbard *et al.,* 2002). In their overall architecture, *tb1* mutants of maize resemble teosinte.

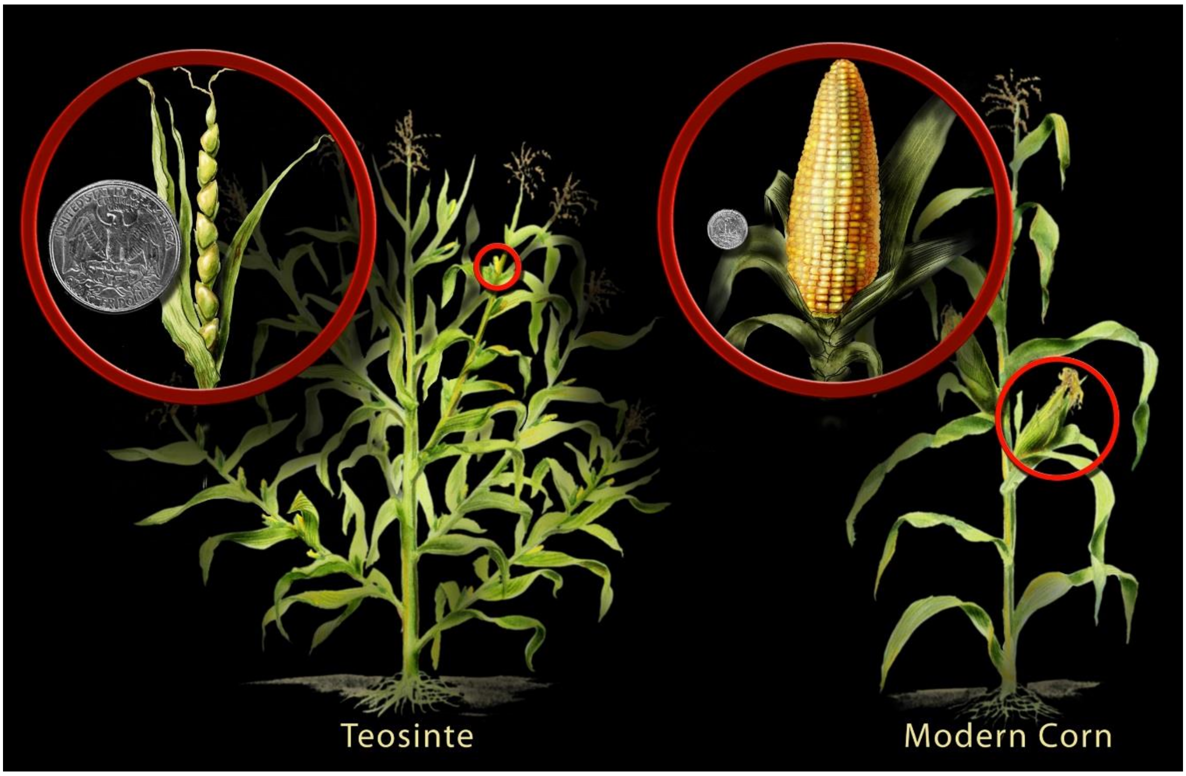

Figure 3. *Comparison of the appearance of adult teosinte and maize plants (©National Science Foundation).*

3.1.3 Differences between maize and other species in the early vegetative stage

While making a distinction between maize and teosinte in the adult (generative) stage is possible, in a maize field crop it is important to discriminate between the two in an early (vegetative) development stage, as teosinte is judged a weed at that point. Discrimination between teosinte and the different grass weed species that may occur is also important, because they require different weed control strategies. Figure 4 (left plate) shows the most important grass species in maize crops that resemble maize in the Netherlands. The species resembling maize in an young stage the most, and which occurs in the Netherlands, is *Sorghum halepense* (Figure 4, right plate). Both *Zea* and *Sorghum* are members of the Andropogonea tribe.

The five in a young stage maize-resembling weed species shown in Figure 4 all have a first true leaf which arrives with a round base, as is also true for maize. Discrimination between maize crop plants and these and other monocotyledon weed species in early development stages is possible by looking at the ligule, auricle and hair (hairy/hairless). The description and differences between these species

in older species can be found in the flora of Heukels (Van der Meijden, 1990). However, maize and teosinte do not differ in this respect, as both have a small ligule and no auricle or hairs on the base of the leaves, as determined from *Z. mays* subsp*. mays* (Figure 5).
According to weed experts investigating occurrences of teosinte in maize fields in Spain, it is not possible to discriminate between young maize and teosinte plants above the ground (A. Cirujeda, Institut Agroalimentario Aragon, pers. comm. 2018). To discriminate, the expert dissects plants and looks at the seed. The seed weight of maize (0.11-0.35 g) is between 5 and 15 times the seed weight of teosinte (0.02 g; Flint-Garcia *et al*., 2009). Apart from differences in shape and size, teosinte seeds are whitish grey, or dark, almost black, with lighter spots, whereas most cultivated maize seeds (in the Netherlands) are yellow, or pink when treated with pesticides (Figure 6).

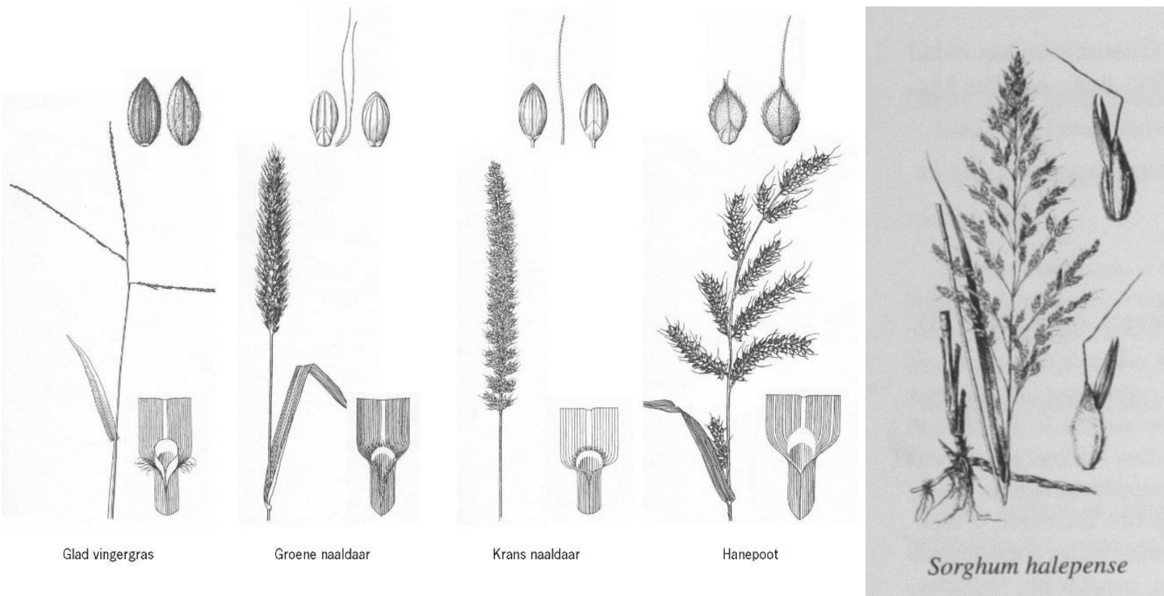

Figure 4. *Left: Important maize-resembling grass weed species in the Netherlands:* Digitaria ischaemum*,* Setaria viridis*,* Setaria verticillata *and* Echinochloa crus-galli *(Handboek Snijmaïs, 2018). Right:* Sorghum halepense *(Hammel* et al.*, 2003).*

Figure 5. *Leaf sheath and emerging leaf in a young plant of (left)* Zea mays *ssp.* mays *and (right) teosinte, from seed labelled* Zea mexicana *(©Wageningen Research).*

Figure 6. *Seeds of (left) teosinte, labelled as* Zea mexicana*, and (top right) treated seeds and (bottom right) untreated seeds of* Zea mays *ssp.* mays *(©Wageningen Research).*

3.2 Occurrence of teosinte

3.2.1 Occurrence of teosinte in Europe

Originally distributed primarily in Mexico, teosinte has dispersed (both naturally and by man) to several countries around the world (see Figure 2). *Zea luxurians* (*Euchlaena luxurians*) from Guatemala seems to be the original source, at least for the distribution by the English and French during the 19th century (Gonzalez *et al.,* 2018). In addition, significant amounts of seed were transported from Egypt and India to the West Indies, Cyprus, South Africa, tropical Africa, Australia, the United States and Guyana (British Guiana). In many of these countries, teosinte is cultivated as a forage crop.

In Europe, however, teosinte is seen as an invasive weed since its first occurrence in Spain and France. In recent publications (Devos *et al*., 2018; Trtikova *et al*., 2017; EFSA, 2016), two main teosinte species are reported in Europe. *Zea mays* subsp. *parviglumis* has been observed in the Poitou-Charentes region of France, since 1990, while a teosinte assumed to be *Z. mays* subsp. *mexicana* has been observed in the Ebro Valley in Aragon, Spain, since 2009 and to a lesser extent in Cataluña since 2014. Trtikova *et al*. (2017) concluded that the latter is of admixed origin, most likely involving *Z. mays* subsp. *mexicana* as one parental taxon and an unidentified *Zea mays* subsp. *mays* variety as the other.

The lower boundary of the mean annual temperature range for *Zea mays* subsp. *mays* (18.04 ± 5.93°C range) is the lowest of all *Zea* taxa, according to data derived from Hufford *et al*. (2012). Similarly to maize, for which temperature boundaries have been extended through breeding (maize is grown in Europe as far north as Scandinavia), teosinte may also be able to adapt to cooler climate conditions. The annual teosinte taxa with mean annual temperature ranges closest to maize, and thus primary suspects for survival in the Netherlands, are *Zea mays* subsp. *mexicana* (16.82 ± 1.98°C range) and *Z. perennis* (16.38 ± 2.16°C range) (Hufford *et al.*, 2012). Furthermore global warming enhances the development of weeds in the Netherlands (Riemens *et al*., 2015).

There is evidence of hybridisation of maize with the teosinte subspecies of *Z. mays* (subsp. *parviglumis*, *mexicana*, *huehuetenangensis*) and with *Z. diploperennis*, and *Z. luxurians* (Tenaillon *et al.,* 2011; Ellstrand *et al.,* 2007; Baltazar *et al.,* 2005; Doebley, 1990; Wilkes, 1967). In general,

according to Andersson & de Vicente (2010), the possibility of hybridisation between maize and most of its wild relatives ranges from moderate to high. The likelihood of gene introgression from domesticated maize to its wild relatives differs between the species and the physical proximity (less than 200 m) and flowering overlap.
Teosintes in Europe act as weeds in maize fields. Their behaviour and fate can be compared with the history of wild oats in cereals. As in wild oats, the period of seed shed (shattering) in teosintes is longer and partly earlier than in the main crop (maize) and control with herbicides in a crop which it resembles in appearance is difficult. The presence of teosinte can cause yield losses in maize of up to 60% (Pardo *et al.,* 2016). In some cases, farmers are forced to plough down their maize crops shortly after sowing due to a high rate of infestation by teosinte and difficulty in controlling it in established maize. In heavily infested fields in Spain, planting maize or sorghum or allowing livestock to graze is banned for three years. Furthermore, teosinte plants growing not only in fields but also in ditches, along road edges etc. must be eliminated and harvesters and other machinery cleaned. This strategy for Spain has been rather successful so far (Alicia Cirujeda, Institut Agroalimentario Aragon, pers. comm. 2018).

3.2.2 Occurrence of teosinte in the Netherlands

No teosinte species at all are listed or reported in the Dutch distribution databases consulted during the present research (www.verspreidingsatlas.nl and www.waarneming.nl). Moreover, a telephone survey of teaching gardens in Amsterdam (13), Utrecht (9), Rotterdam (16) and The Hague (18) and 2 out of the big cities did not yield positive reports of teosinte cultivation. This is despite the fact that Vreeken's Zaden claims that it has sold around 100 batches of teosinte seeds every year for about a decade, mainly to teaching gardens in the Netherlands (Ton Vreeken, Vreeken's Zaden, pers. comm. 2018). Providing that the seeds sold are actually sown and cultivated, the lack of reports on teosinte occurrence hints at no further natural spread.
The poster presentations and subsequent spread of information through several media in autumn 2017, together with the email consultation in Spring 2018, reached the majority of agricultural advisors and crop protection industry experts and over 100 maize growers in the Netherlands. In this major outreach, agronomic experts were prompted to report any unknown or unidentified weeds which could possibly be teosinte. in total three reports (series of images) were made of unknown or unidentified weeds, but none of these was teosinte, as determined by Wageningen University & Research weed scientists and Ton Rotteveel, retired taxonomist. Especially *S. halepense* can be mistaken for teosinte.

3.3 Feral maize

3.3.1 Definition regarding maize going wild

For the purposes of Environmental Risk Assessment (ERA) of genetically modified (GM) plants, parties applying for a licence for market approval are required to provide information on whether the crop species in question can form volunteer populations in arable fields, form feral populations in disturbed semi-natural areas or even invade natural areas (Kos *et al.,* 2011). This is important with regard to possible contamination of natural habitats with GM material and the contribution to the total adventitious GM content of the non-GM final product (Pascher, 2016). In Austria, data on the spread of volunteer and feral maize plants were reported recently (Pascher, 2016). However, it is unclear whether the definition of Gressel (2005), by which crops must establish and persist outside arable fields in order to be called feral, was used in that work.
An update on the questions of whether maize can form self-sustaining populations in (semi-)natural environments, whether it can form feral populations and the extent to which it forms volunteers in the Netherlands is needed and is being sought by COGEM. In the first instance, clarity on the terms volunteer plant and feral population are needed to enable proper analysis and discussion of the situation. These are defined as follows in general and in this report:

- A *volunteer plant* is a plant that has not been deliberately planted (Oxford Dictionaries; https://en.oxforddictionaries.com/definition/volunteer). In weed management, volunteer crops are leftovers from earlier cultivation.

- In this report we define a volunteer population as a group of plants emerging unintended in an arable environment (field) based on leftovers from earlier cultivation in that field.

- *Feral* means “in a wild state”, especially after escape from captivity or domestication (Oxford Dictionaries; https://en.oxforddictionaries.com/definition/feral).
- *Feral* according to Heukels’ Flora (23rd ed., 2005) comprises cultivated plants that grow outside fields and cultivated plants that have maintained after re-designation of their growing site.
  - In this report we define a feral population as a group of plants in a semi-natural area that arrived at that place unintended. A feral population may either survive for a limited period or develop into a self-sustaining population.
  - In this report we define a self-sustaining population as a group of plants that germinate, emerge, survive, produce new seeds, shatter these seeds naturally and produce new seedlings and plants for three years without human intervention.

3.3.2 Factors influencing maize going wild

The likelihood of a crop forming a feral or self-sustaining population depends on plant traits, while other factors best predict the regional and local frequency of invasiveness of exotic plant species (Speek *et al.,* 2011). The regional frequency indicators are height, flowering season length, polyploidy level, origin (European vs. non-European) and life form (hemicryptophytes, geophyte, phanerophyte). The local dominance indicators are life form, period of introduction and vegetative lateral spread. Also Warwick & Stewart (2005) list domestication characterisation properties for use in discriminating between domesticated species, weeds and invasive species. Kos *et al*. (2011) gave small seeds and having a seedbank (dormancy) as indicator for weeds or invasiveness.

Anderson and de Vincente (2010) review the invasiveness and domestication characteristics of crop species. Regarding maize, they point out that maize pollen is released in very large quantities over a relatively short period of 5 to 20 days, depending on climate zone. In improved maize lines, flower development, pollen shedding and fruiting are synchronised and overlap markedly. Domesticated maize does not reproduce vegetative. Moreover, during domestication maize lost its ability to disperse seed, and thus to survive in the wild. Its seeds (kernels) remain on the cob after ripening, and do not shatter or otherwise scatter naturally. Maize cobs, or parts of cobs, can remain in the field and form volunteers, and can germinate, grow and develop in a feral way, but it is considered unlikely that they will successfully establish feral populations beyond arable fields and without human support (Trtikova *et al*., 2017). Even if small amounts of maize seed, or whole cobs, are distributed by humans and animals, maize seeds dispersed during the harvesting process can only survive for up to one year in the soil, due to their poor dormancy. Furthermore, domesticated maize requires warm conditions in order to grow and does not tolerate periods of cold and frost. Therefore maize seeds and their seedlings have a poor chance of survival in many (European) maize-growing regions. Even in regions where winter temperatures are not low enough to kill off volunteers (e.g. Mediterranean countries), maize volunteers and feral plants do not form self-sustaining populations because lack of scattering and seed dormancy and poor competitive ability in the natural environment. Hence, cultivated maize, which its big seeds and no seedbank, can generally be considered to pose little to no risk of becoming a volunteer or feral weed.

Van der Wiel *et al.* (2011) performed a literature review on the relationship between volunteer occurrence of three crop species (maize, sugar beet and potato) and climate conditions, and compared the results with the four climate scenarios for the Netherlands by 2050 made by the Royal Netherlands Meteorological Institute (KNMI). They concluded that the important climate factors for volunteer occurrence are: 1) soil moisture conditions after harvest, since these influence volunteer seed depletion by germination and subsequent removal by winter conditions or control measures (maize and beet) and seed dormancy development (beet); 2) frost conditions during winter, which determine survival of reproductive plant parts, particularly groundkeepers (beet roots and potato tubers); and 3) temperature and soil moisture conditions during the growing season, which are important for growth and reproduction of volunteers. According to Van der Wiel *et al.* (2011), maize does not show volunteerism in the Netherlands, even with the already ongoing warming of the climate.

3.3.3 Occurrence of feral maize in the Netherlands

For two Dutch databases on the distribution of species in the Netherlands (Waarneming.nl and Verspreidingsatlas.nl), the number of observations of maize compared with the number of observations of other crops and several weeds are shown in Table 2. The distribution database www.verspreidingsatlas.nl is produced by officials observing species in 1-km^2 grid cells by professionals (Floron, 2016) and other observations of several sources are added too (personal communication Ruud Beringen, Floron, 2018). Arable fields and gardens are not observed for this database, so the numbers should reflect appearance in (semi-)natural conditions. In comparison with the other cultivated crops listed, the numbers for maize are relatively low, particularly in respect of the area of the crop grown in the Netherlands (~215,000 ha in 2018) compared with e.g. potatoes (c.120,000 ha in 2018). Maize is mainly grown as fodder crop (silage maize) in the Netherlands. Some maize seed is also sold as an ingredient of pet and bird feed.
The email inventory in September resulted in very limited positive responses. Two responses reflected an impression of possibly increased presence of volunteer maize plants in fields in crops over recent years. No actual data was supplied supporting these impressions. In the responses received no indication at all is reflected of feral or self-sustaining maize populations.

Table 2. *Yearly number of observations of species in the Netherlands within the grids of the species distribution database www.verspreidingsatlas.nl (years 1990-2018) or as single reports in www.waarneming.nl (years 2013-2017).*

Species	Name	Verspeidingsatlas.nl	Waarneming.nl
Zea mays subsp. mays	Maize	195	15
Beta vulgaris subsp. *vulgaris*	Sugar beet	183	145
Solanum tuberosum	Potato	445	39
Triticum aestivum	Wheat	502	46
Brassica napus	Oilseed rape	1321	46
Brassica rapa subsp. *oleifera*	Rapeseed	1192	417
Solanum nigrum	Sticky nightshade	1630	491
Echinichloa crus-galli	Barnyard grass	1580	686
Matricia maritima ssp. *inodora*	Scentless mayweed	1626	859
Poa annua	Annual meadowgrass	1661	1641
Stellaria media	Common chickweed	1663	1666

The reports for the crowd-sourcing website www.waarneming.nl are made by volunteers and not necessarily systematic every year. For these reports, exact locations, numbers and often pictures are included in the database. To get an impression of the current situation and whether there were self-sustaining populations the 15 reported sites of maize in that database (Table 2), any location that had more than four plants reported in the last three years (which amounted to three locations) was visited in mid-August 2018.

At the location in Amsterdam reported in 2015, the seeds seem to be deliberately sown. The location is adjacent to a canal in which houseboats are moored, making the plant location a “front yard” for their residents. Figure 7 shows the situation at the location in 2015 and in 2018 (Figure 7).

2015 2018

Figure 7. *Comparison of a reported location of maize in Amsterdam in 2015 with the same site in 2018, where sunflowers (*Helianthus annuus*) grew.*

During the revisit of the location in Figure 8 in Amsterdam reported in 2016, in August 2018, no maize plants were found. The pictures in Figure 8 show the situation for 2016 at the location, which is a combination of housing and industry.

Figure 8. *Left and Right: Reported location of maize in Amsterdam in 2016. No maize was present at the site in 2018.*

In 2017 and 2018 two situations were reported in Almere. No pictures were available of these situations. The 2017 and 2018 locations were very close to each other (within 300 m) and designated building sites. The report made in June 2018 appeared to be a maize field in August, so the crop was probably sown late which assumed to be groups of volunteer plants

In autumn 2018 in some arable field volunteers of maize could be found (see figure 9). It was easier to find this volunteers in this year because of the relative early harvest of maize and the warm autumn period resulting in better growing conditions and bigger plants which can be found more easy. This illustrates the lack of dormancy and emerging of volunteers before winter and before a new cropping season resulting in no volunteers in the crops after the winter.

Figure 9. *Left and Right: Volunteer maize plants in an arable field in Vaassen in autumn 2018 at 24 October, and after frost at 14 November.*

3.4 Evaluation approach followed and future prospects

To try and find possible teosinte and feral maize in an efficient way, without extensive physical search activities, existing networks and databases were used maximally. As far as presence of maize and teosinte outside field concerns, the data from Verspreidingsatlas.nl are generated by educated officials and give a good indication of spread over time. The data of Waarneming.nl is additive to that and has the benefit of supplying pictures, which in many cases are very illustrative; these pictures possibly (partly) reflect the situations in which maize plants are found that constitute the data gathered in Verspreidingsatlas.nl.
Expectations are that teosinte, in case it spreads, first will be found in arable maize fields. Teosinte will escape chemical control with herbicides selective for maize resulting in less competition compared to natural surroundings. Therefore searching for teosinte in agricultural areas seems an appropriate method. Creation of awareness within the agricultural community was effective in the way that reports came back of unknown weeds (which in all cases was not teosinte). This was both the result of the poster presentation and of the email consultations among key experts and advisors on arable farming and maize cultivation.
The pathway of searching teosinte plants through contacting possible buyers of teosinte did not result in finding actual (teaching) gardens growing the plants, despite approaching over 50 gardens. This route may have been hampered by the privacy legislation taking effect in Spring 2018, forbidding sharing personal information with third parties.
Evaluating the approach, the chances of finding teosinte were used maximally within the framework of the assignment, and authors are convinced teosinte would have been found if present in arable fields, even in a fairly low frequency. To a great extent the same goes for feral maize. Self-sustaining populations are claimed to be absent and proof otherwise was not found; feral populations do exist, in terms of one or more plants that occur outside a field in a single year.
Maize volunteer plants are observed. Experts responding in the email consultations have the impression of increasing frequency over recent years, but to date there is lack of more precise data.

4 Conclusions & recommendations

4.1 Teosinte occurrence

No indication was found that teosinte has spread in the Netherlands, other than through seeds sold commercially. The network consultations performed within the framework of this COGEM assignment yielded no reports of teosinte in maize cultivation areas, although the vast majority of maize experts and advisors in the Netherlands were reached. Even the reported sale of around 100 batches of teosinte seed by Vreeken's Zaden annually did not result in positive reports of cultivation of teosinte in the Netherlands in 2018.
Future occurrence of teosinte in the Netherlands could be limited by banning the sale and use of teosinte seeds (including use in pet and bird feed), although it can be discussed whether this is needed. . A proposed monitoring strategy could follow three paths: 1) following up on seed sold, e.g. by adding an instruction leaflet with teosinte seed batches asking the buyer to report any natural propagation of teosinte sown; 2) monitoring the weed situation in farmers' fields, by increasing farmers' knowledge of teosinte and/or increasing their awareness of new weed species; and 3) by interviewing weed control experts on a regular basis.

4.2 Self-sustaining and feral maize populations

Several publications state that maize as a species cannot form a self-sustaining population under (semi-)natural conditions. Even in southern parts of Europe with mild winter conditions, there are no reports of self-sustaining maize populations. Moreover, based on the data in the present report, there is no indication that the current situation in the Netherlands is different, as there have been no reports at all of possible self-sustaining maize populations. This makes the risk of development of self-sustaining populations in the near future very unlikely.
According to several publications, even feral maize populations and volunteers are scarce and this situation is not expected to increase much, even not with climate change. However, the email consultation rounds give food to the perception among farmers and advisors that maize volunteer numbers may be rising. Unfortunately, empirical data on this issue are lacking. This data gap could be overcome through applying the following steps: 1) farmers and advisors could be questioned and/or invited to make counts, gathering qualitative or indicative quantitative data; 2) to obtain solid quantitative data, some kind of scientific monitoring programme could be implemented; and 3) the Dutch National Database Flora and Fauna biologists could be instructed or invited to make observations of maize volunteers in fields.
A similar approach can be recommended regarding feral maize. According to the definition we used (see Section 3.3.1), feral includes volunteers outside arable fields, so a more structured approach in terms of marking plant locations is needed than when recording volunteers within fields. It is crucial to determine whether plants return at the same location for consecutive years. If this is to be monitored by Dutch National Database Flora and Fauna biologists, the assessment method used needs to be standardised and also marking exact locations. Alternatively, people reporting maize occurrences on www.waarneming.nl could be asked to re-visit sites where they reported maize in the previous year and report on repeated maize presence; also COGEM could organise revisiting or alternative monitoring.

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Annex 1 Poster presentation

WAGENINGEN
UNIVERSITY & RESEARCH

GEZOCHT!
Teosinte (oermaïs) en maïs buiten de percelen

Achtergrond

In het zuiden van Frankijk en in Spanje hebben maïstelers last van "teosinte" of "oermaïs" als een onkruid in de maïs. Deze oermaïs heeft kleinere zaden die eerder en makkelijker uitvallen en verwilderen. Omdat de plant zo op maïs lijkt is hij lastig te bestrijden in het gewas maïs. Vroege signalering van de aanwezigheid van teosinte is belangrijk om verdere verspreiding te voorkomen.

Verder meldt men in centraal Europa opslagmaïs en maïs dat overleeft buiten de akkers. Tot nu toe wordt ervan uitgegaan dat maïs zich in Nederland niet kan handhaven buiten de akker.

In opdracht van de COGEM (Commissie Genetische Modificatie) wordt een inventarisatie uitgevoerd in Nederland naar teosinte en verwilderde maïs. Dit is belangrijk voor de milieu-risicobeoordeling die uitgevoerd wordt bij de import van GMO maïs.

Wat kunt u doen?

Foto | teosinte | © Elizabeth M. Hoover |

Weet U een plek waar oermaïs groeit of ziet U maïsopslag of maïs dat zich buiten de akker staande weet te houden, maakt u dan een foto en stuur deze met de lokatie van de vindplaats en uw contactgegevens naar

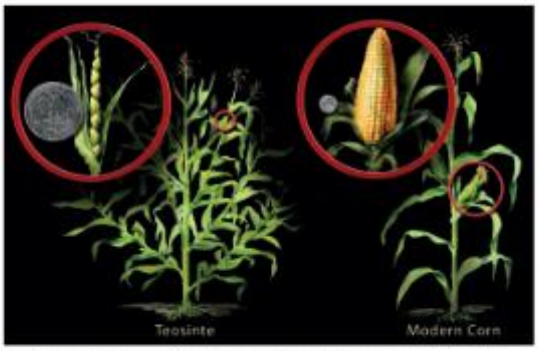
info.ruwvoerenbodem@wur.nl

Gezocht:
De eerste 10 melders van verwilderde teosinte of verwilderde maïs ontvangen een cadeaubon.

Wat is teosinte (oermaïs) – hoe te herkennen?

De domesticatie van maïs begon zo'n 5000 jaar geleden, ruim voordat de maïs door Columbus naar Europa werd gebracht. Teosinte wordt algemeen gezien als de (een) wilde voorouder van de huidige maïs. Eigenlijk omvat de term teosinte het geheel aan soorten of typen oermaïs. De bekendste zijn *Zea mays* ssp. *mexicana* en ssp. *parviglumis*.

Net als maïs is teosinte een C4-gewas en kortedagplant, maar in vergelijking met maïs lijkt teosinte meer op een fors uitgevallen gras; de planten zijn korter, meer vertakt en de zaden bevinden zich in een dunne aar. Ook zijn de zaden in vergelijking met maïs een stuk kleiner.



Figuur | vergelijk teosinte en maïs | © National Science Foundation |

Foto's | maïs buiten de akker of opslag | © Katrin Pasher 2016 |

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Report WPR-3750364300

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The mission of Wageningen University and Research is “To explore the potential of nature to improve the quality of life”. Under the banner Wageningen University & Research, Wageningen University and the specialised research institutes of the Wageningen Research Foundation have joined forces in contributing to finding solutions to important questions in the domain of healthy food and living environment. With its roughly 30 branches, 5,000 employees and 10,000 students, Wageningen University & Research is one of the leading organisations in its domain. The unique Wageningen approach lies in its integrated approach to issues and the collaboration between different disciplines.