Plant material inactivation

How to eliminate seed and plant lots commingled with non-authorised GM material

CGM 2020-05

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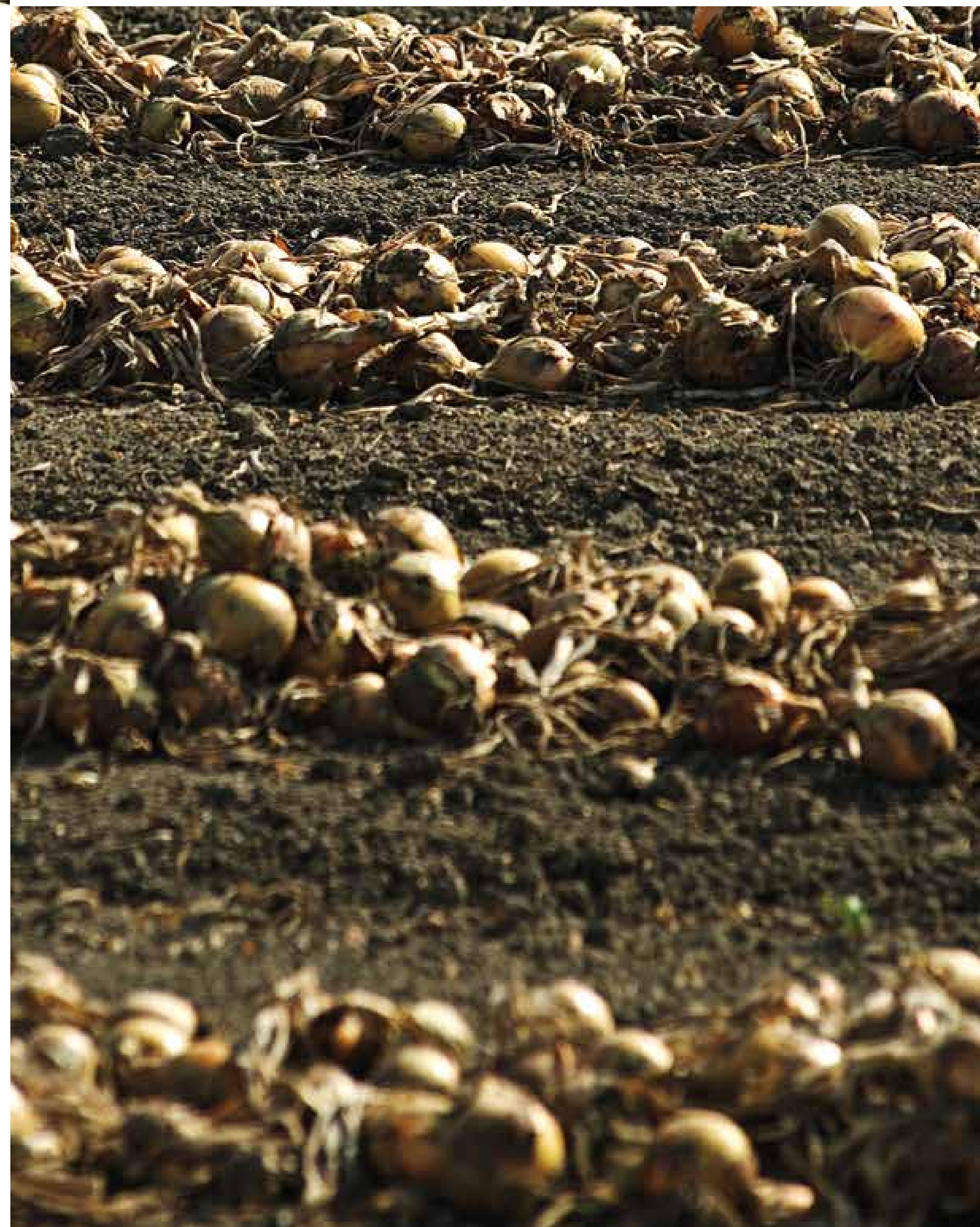

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COGEM Report CGM 2020-05

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The authors gratefully acknowledge the members of the advisory committee for the valuable discussions and patience.

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- Dr. Peter Bruinenberg, AVEBE Plant breeding
- Ir. Nicolas De Schrijver, Perseus, Biosafety and Regulatory Specialist
- Ing. Martin van Dam, Wageningen University & Research, researcher flower bulbs

Disclaimer

Foreword

In recent years, there have been a few incidents with non-authorised genetically modified (GM) crops. Petunia varieties were found to be genetically modified, and seeds of a GM crop (maize, soybean or oilseed rape) were detected in conventional seeds for sowing. In two cases the seeds were already sown before the presence of a non-authorised GM crop was detected.

In such cases, the Human Environment and Transport Inspectorate which monitors and enforces the GMO legislation, asks COGEM how the GM plants can be destroyed. The Human Environment and Transport Inspectorate wants to prepare itself for future incidents with non-authorised GM crops, and therefore it asked COGEM to provide advice on the destruction of non-authorised GM crops in general.

To prepare for this advice, COGEM commissioned Perseus B.V. to write a report on the methods which can be used to destroy plant material of different crops in all possible growing stadia (sowing seeds, emerged or fully grown plants etc.).

Perseus gathered information on methods to control weeds, and on methods to destroy plant material that may contain quarantine organisms. In addition, protocols that describe how to destroy GM plants were consulted and several persons with experience in the removal of GM plants after field trials were interviewed. In the resulting report the pros and cons of different inactivation methods are described and guidance on the inactivation method that is most suited to destroy crops in their different growth stages is given.

The steering committee enjoyed the interaction with the researchers during the physical and digital meetings, and is pleased with the report. It is confident that it will be of value in case of any future incidents with non-authorised GM crops.

Dr. Ir. Rommie van der Weide
Chair of the Advisory Committee.

Summary

The inadvertent presence of non-authorised GM events in plant materials destined for sowing or planting or that is already in the field may trigger a decision that the material must be inactivated. In the Netherlands the Ministry of Infrastructure and Water Management - Human Environment and Transport Directorate (ILT) is responsible for monitoring imported plants and seeds for sowing for unauthorised presence of GM events, and enforcement, amongst others. Also, companies, organisations and other authorities may notify the ILT of the presence of unauthorised GMOs on Dutch territory.

Several inactivation methods are available, such as composting, ploughing, herbicide spraying, incineration. Depending on the species and growing stage, one or the other method is more suitable. In order to have a ready-to-use guideline, this report inventories and classifies inactivation methods in relation to the plant species and development stage.

Adventitious presence of non-authorised GM events not only originates from events authorised for commercial release elsewhere in the world, but also from inadvertent commingling of GM material during research and development (Bashandy and Teeri, 2017) and field trials. In this study an inventory of species for which GM events are known that have reached this stage was made, confirming the broad botanical range with nearly 160 species. A selection of species that are relevant for the Netherlands was further classified in 13 categories based on type of life cycle, winterhardiness of the plant and its survival structure(s), survival/dissemination structure, secondary seed dormancy, pollination method, presence of sexually compatible species, seed shatter, and potential to form feral populations.

At the same time, inactivation methods were identified and described with a specific focus on applicability, advantages and shortcomings. They were grouped according to the primary mode of action (physical, biological, mechanical or chemical). Since only few references are specific for GM plants, inactivation methods that are used to combat weeds in general were referred to where available. On the other hand, weeds are not completely comparable to domesticated crops as weeds may be more resilient. In consequence, GM crop species may be controlled with less stringent methods. Finally, scientific literature presents diverging efficacy figures on some methods which may be related to the effect of environmental conditions.

The adequacy of the different methods for inactivating the different GM plant categories was made, linking information on the biology of the plants with the applicability and efficacy of the inactivation methods. A decision tree (Figure I) is proposed as well as a summary table A that helps to identify the most suitable methods depending on the type of material and/or stage of development. While these summaries can provide a quick overview, readers are invited to verify the details in the tables of Chapter 6.

An additional category includes unclassified species covering both species not described in any of the above listed categories and species for which so far no GMOs have been released in the environment. They can be assessed taking into account the characteristics as mentioned in Chapter 2 and comparing the classification in Chapter 3. If no information on the biology can be obtained or the situation urges for quick action, the most drastic inactivation methods can be applied (e.g. incineration, herbicide treatment).

Figure I – Summary decision tree to inactivate plant materials according to plant material type and the developmental phase (blue boxes indicate information, yellow – decision questions, green – inactivation options, most practical/effective solutions are indicated in **bold;** compatible species = sexually compatible species; isol. dist.= isolation distance, ⊕ = options)

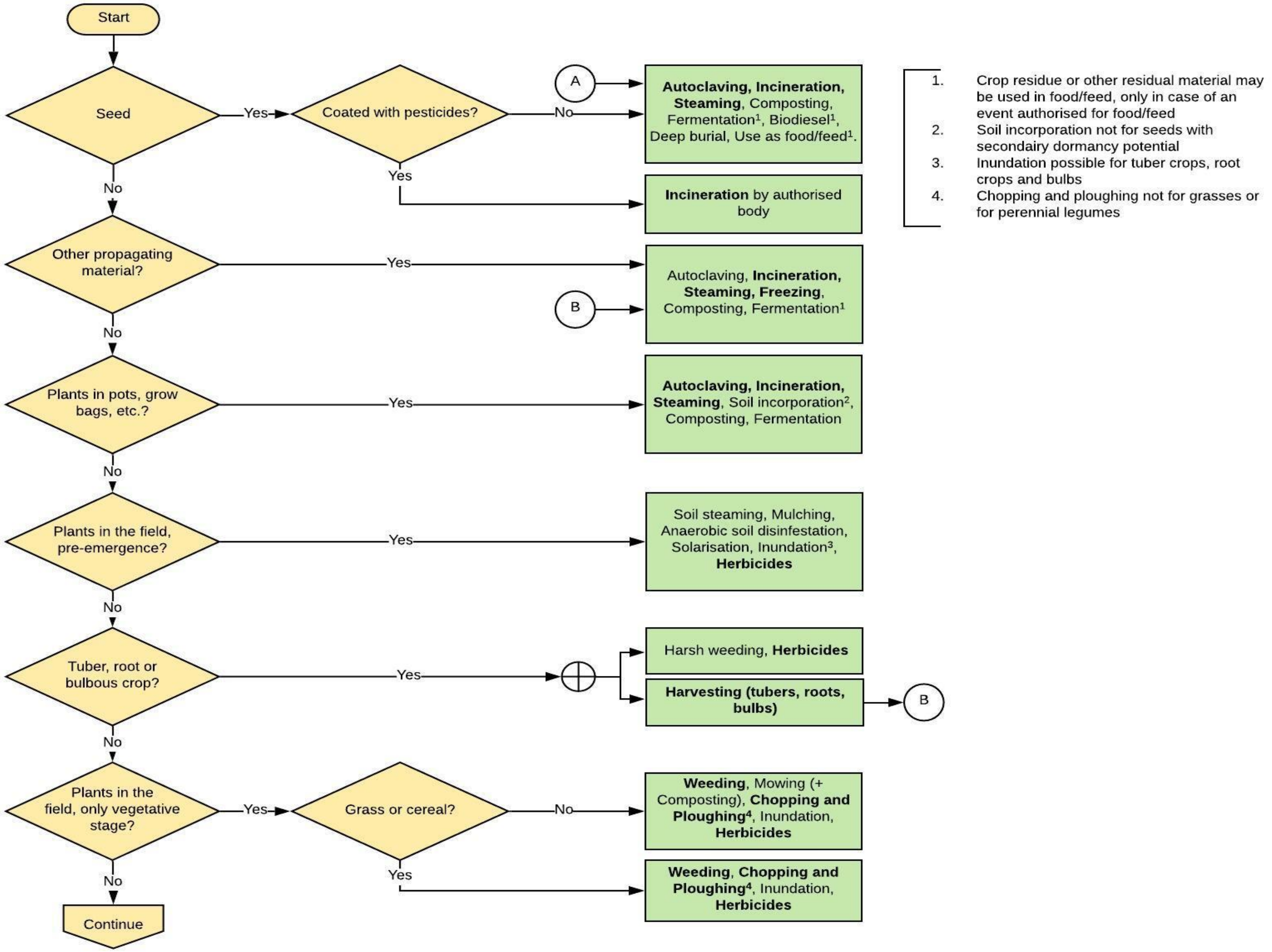


Figure I – continued

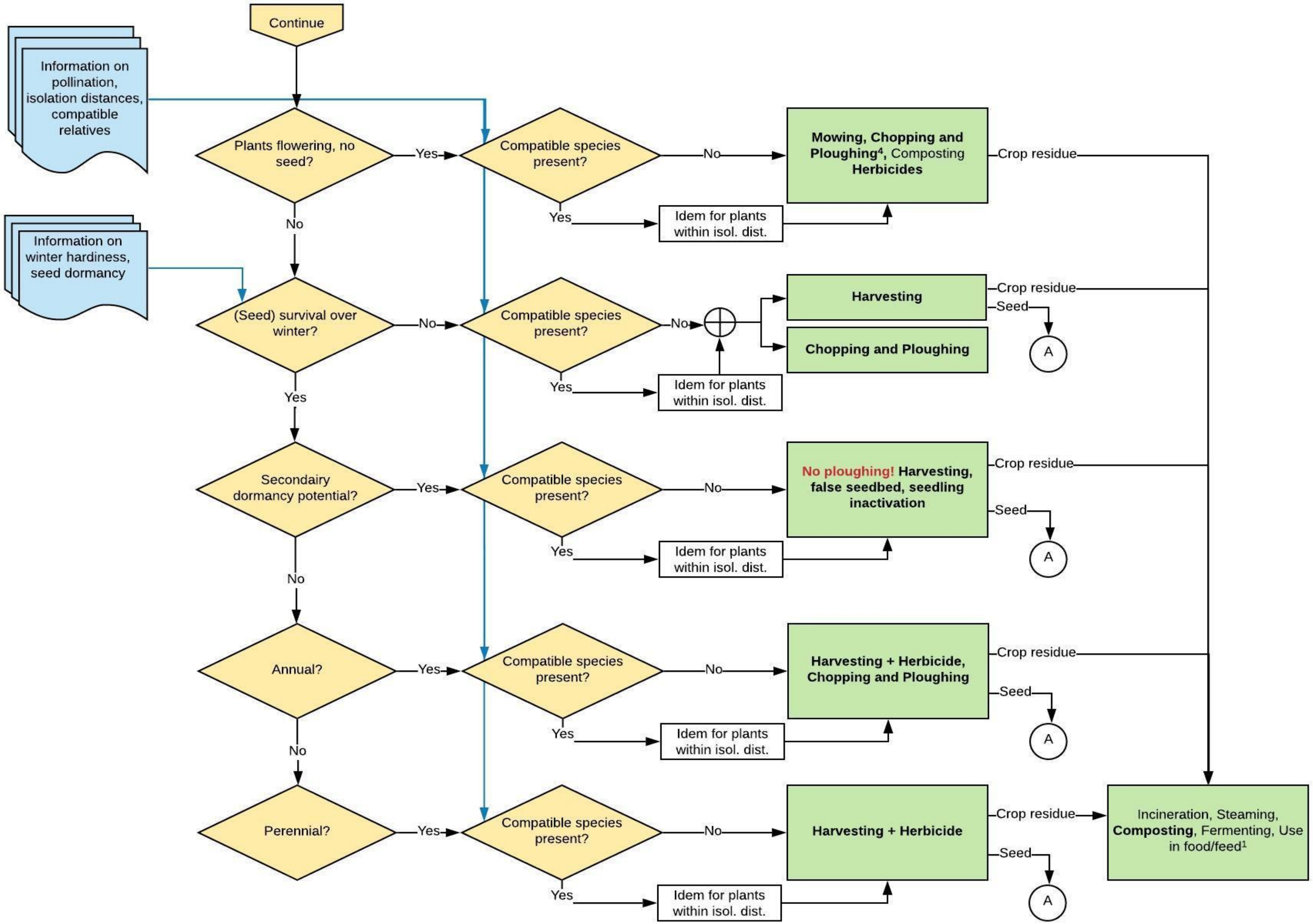


Table A – Summary table of most suitable inactivation options per category and type of material (as relevant)

	Grasses	Winter hardy cereals	Non-winter hardy cereals	Crucifers and species with similar characteristics	Annual legumes and species with similar characteristics	Perennial legumes	Tuber crops	Root crops	Annual fruit & vegetable species (non-winter hardy)	Bulbs	Pot and bedding plants	Trees
Seed	S/ A / I	S/ A / I	S/ A / I	S/ A / I	S/ A / I	S/ A / I		S/ A / I	S/ A / I	S/ A / I	S/ A / I	S/ A / I
Coated seed	I	I	I	I	I	I		I	I	I	I	
Tubers							F/ S					
Seed sown, not emerged	PeH	PeH	-	PeH	-	PeH	-	PeH	-	PeH		
Seeds sown, seedlings and young plants in growing medium				S/ A / I					S/ A / I	-		S/ A / I
Seedling and rosette stage				MeW / C/ D/ H					-	-		
Vegetative phase / Bolting stage (soil bound)	MeW / H	MeW / D+P / H	MeW / D+P / H	H	MeW / C/ D/ P / Mow/ H	MeW / H	H	MeW / H	Mow/ D/ C+P	H	Uprooting	Sch+
Vegetative + generative phase (soilless/ pots)									S/ A / I	S/ A / I	S/ A / I	
Flowering	M+H	C+P	C+P / Ha	Ha	C/ D/ P / Mow/ H	Mow+H/ H	Ha	Pu+D	Mow/ D/ C+P	H or S/ A / I	Uprooting or S/ A/ I	
Seed set	M+H	Ha+	C+P / Ha	Ha	C+P / Ha	Mow+H	Ha	Pu+I	Mow/ D/ C+P	H or S/ A / I	Uprooting or S/ A/ I	
Tuber/ root/ bulb stage							Ha	H+CP		H or S/ A / I		
Spilled seed	G+H/MeW	P / G+H/MeW	-	G+H/MeW	-	G+H/MeW	-	G+H/MeW	-	-		
Aftercare	Mon	-	-	Mon	-	Mon	Mon	Mon	-	Mon		

(Abbreviations: A = Autoclaving; C = Chopping; C+P = Chopping and ploughing; D = Disking; D+P = Disking and ploughing; G+H = allow germination and herbicide treatment; F = freezing; Ha = Harvest; Ha+ = Harvesting + treatment (disking, ploughing or herbicide); H = Herbicide treatments; H+CP = Herbicide treatment followed by chopping and ploughing; I = Incineration, M+H = Mowing + Herbicide; MeW = Mechanical weeding; Mon = Monitoring + inactivation; Mow = Mowing; PeH = Pre-emergence herbicides; P = Ploughing; Pu+De = Pulling followed by desiccation; Pu+I= Pulling followed by incineration; Sch+ = Schredding followed by composting, fermentation or use as wood chips; S = Steaming)

Notwithstanding the specific indications per category, the following conclusions can be drawn:

- With the exception of autoclaving and incineration, no method inactivates seeds and plants for 100%.
- Seeds of all species can be treated (almost) the same way: incineration is always adequate, next to steaming and autoclaving. For tuber crops, freezing is an additional valid option.
- Wet biomass can always be composted or fermented, but care must be taken when the material (potentially) contains seeds. In that case it is important to maintain the required high temperature for a sufficiently long period of time. Steaming is also a feasible method, in particular when other material than biomass (pots, soil, etc.) is present.
- In the field, the use of a suitable herbicide to inactivate a crop is the most practical option, but the choice of the herbicide must take into account that the GM may have been modified to be tolerant to specific herbicides. When applied correctly (development stage, dose, weather conditions), it will give a high inactivation efficiency. However, other methods are available when farmers choose not to use chemical plant protection products (farmers wishing to minimise the use of pesticides, organic farmers). Mechanical weeding is the most obvious alternative, next to ploughing (incorporation of the biomass into the soil). In view of the principles of recycling and recovery, the options of mowing or harvesting and collection of the seed and/or the crop residue, the options of composting, fermentation (biogas and ethanol production), biodiesel production and use as or in food/feed in case the commingling GM event is authorised to, are valuable alternatives.
- Inundation, soil steaming, solarisation, mulching and biological disinfestation of the soil remain options, but have disadvantages (e.g. long treatment period) and will usually be considered impractical.
- Whereas farmers will probably like to resume their business as soon as possible, they will choose for short duration inactivation methods, if left the choice. Often this will involve the use of herbicides with short carry-over effect or incorporation of the plant material into the soil, also because machinery is on hand. Mulching, anaerobic soil disinfestation, inundation, soil solarisation may result in missing a growing season. The choice of the following crop should allow for monitoring and inactivation of volunteers. This may involve a change in the usual rotation plan.

In addition to the technical aspects of the inactivation techniques, some general observations were highlighted through the limited experience with previous cases and interactions with the persons involved:

- Identification and quantification of the commingled GM material is required to perform a risk assessment. If it concerns GM plants that have been authorised for import as viable material for processing and food and/or feed use in the EU, some of the environmental risks[1] have already been determined and other handling options (such as direction for food and/or animal feed) may be allowed.
- A key objective is to intervene before spatial dispersal (via pollen to compatible crops) or temporal dispersal (establishment in the seedbank) occurs. For crops that are seeded, such introduction will already occur as of sowing and there always remains a chance that some seeds did not germinate in the initial period.
- The applicability of certain methods is determined by the availability of expensive equipment and suitable facilities. An autoclave may be appropriate for inactivating small quantities, yet this may not be compatible with large batches of soiled material. Furthermore, certain types of installations (e.g. biodiesel or ethanol production plants) may be specialised in routine handling

[1] Although an environmental risk assessment will be a prerequisite for an authorisations for import of viable GM plant material, the scope may be significantly different: for import the focus will be on spillage during transportation, whereas commingling may result in the presence in farmers' fields in optimal cultivation conditions. The relevance of the evaluation for import will therefore need to be considered on a case-by-case basis.

of certain materials and may not be open to receive an occasional diverging batch. In particular if this generates by-products for other sectors (e.g. feed) this may create issues for segregation of products related to authorisations and labelling.

- Ideally, inactivation occurs as close as possible to the site where the material is discovered. However, the most efficient inactivation methods may require transportation. During transportation care must be taken that no material is lost. The discrepancy between the indications of the ADR legislation and the Regeling GGO need to be sorted out in this respect (packaging instructions).
- Monitoring can be implemented to confirm the efficacy of the inactivation or provide a tracking system of remaining problems. Its usefulness must be determined on a case-by-case basis taking into account the species, developmental stage, level of commingling, rotation history and whether the commingling GM event has been authorised for food, feed and processing.
- When GMO presence is detected that leads to an obligation to remove the material, in addition to identifying suitable methods, the responsible parties (including inspection), the type of verifications and the type of expected reporting can be indicated more explicitly. A documented process and follow-up would be useful to track compliance and to learn on best practices.

In spite of the large, global scale deployment of GM plants, the number of cases that have been reported and that required inactivation of plant material remains very limited. However, as more countries are introducing plant biotechnology applications and product authorisations continue to be unsynchronised, this situation is not expected to become less demanding. Furthermore, once an unapproved GMO presence is detected, the impact can be far reaching and fast action is required. As techniques develop, more adequate options may become available. The approach proposed in this study, justifying the choice of inactivation method on biological features of the GM species, provides a framework for future cases.

Samenvatting

De onbedoelde aanwezigheid van niet-toegelaten ggo's in zaai- en pootgoed of in gewassen die al op het veld staan, kan aanleiding zijn om te besluiten dat het materiaal moet worden vernietigd. Het Nederlandse Ministerie van Infrastructuur en Waterstaat - Inspectie Leefomgeving en Transport (ILT) is verantwoordelijk voor o.a. het monitoren van geïmporteerd zaai- en pootgoed op aanwezigheid van niet-toegelaten ggo's en handhaving. Daarnaast kunnen bedrijven, organisaties en andere autoriteiten het ILT op de hoogte brengen van niet-toegelaten ggo's op Nederlands grondgebied.

Er bestaan diverse inactivatiemethoden, zoals composteren, onderploegen, herbicidetoepassing, verbranding. Afhankelijk van de plantensoort en ontwikkelingsstadium, is de ene of de andere methode meer geschikt. Dit rapport heeft tot doel een kant-en-klare handleiding aan te reiken, waarin inactivatiemethoden zijn geïnventariseerd naargelang de plantensoort en ontwikkelingsstadium.

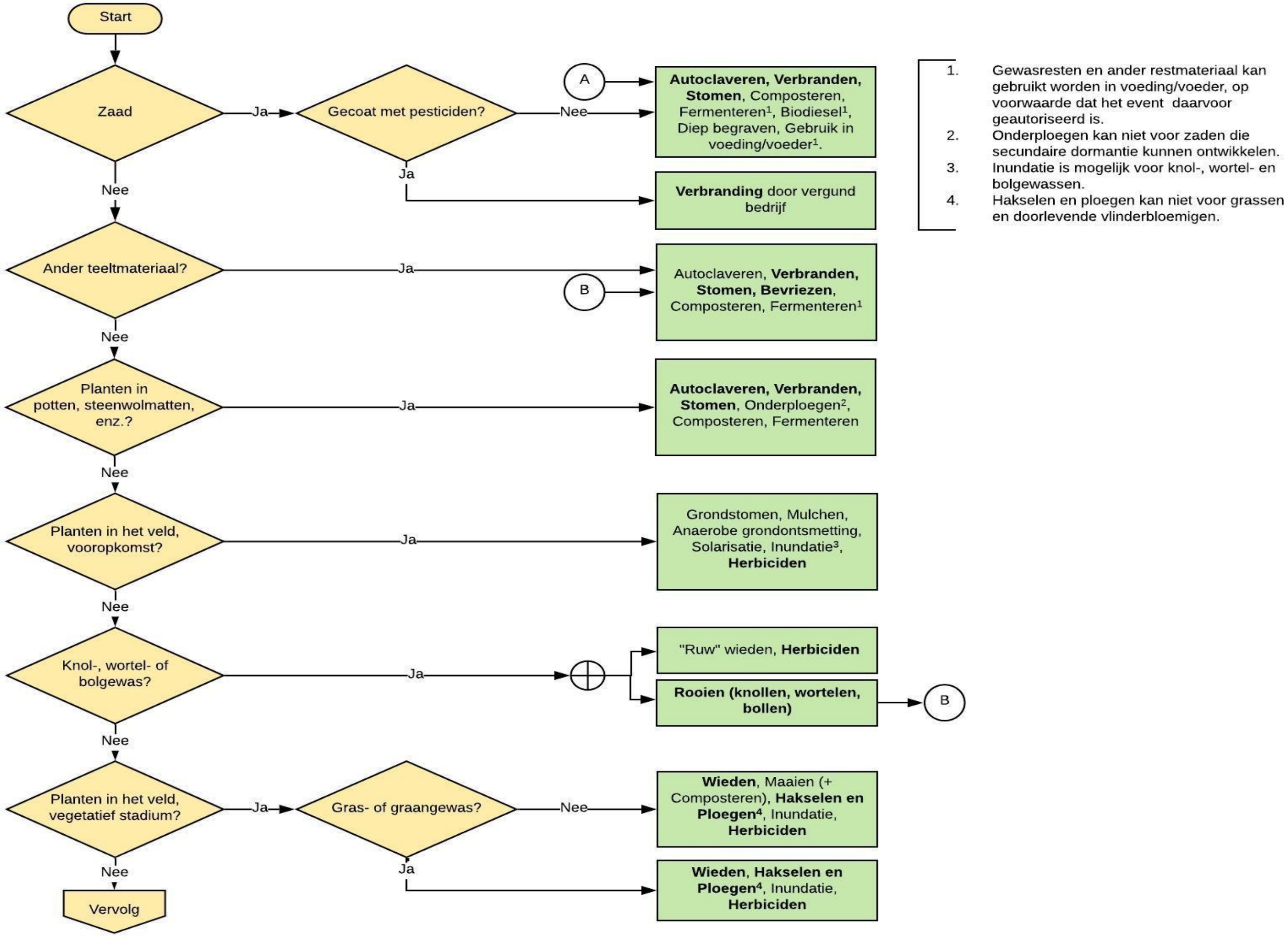
De onbedoelde aanwezigheid van niet-toegelaten ggo's vindt zijn oorsprong niet alleen in ggo's die commercieel toegelaten zijn elders in de wereld, maar ook in het onbedoeld vermengen van ggo's in onderzoek en ontwikkeling (Bashandy and Teeri, 2017) en in veldproeven. In deze studie werd een lijst opgesteld van soorten waarvan gg-varianten gekend zijn in bovengenoemde gevallen. Bijna 160 soorten werden gevonden, wat duidt op een brede botanische reeks. Hieruit werden soorten geselecteerd die relevant zijn voor Nederland en deze werden verder ingedeeld in 13 categorieën volgens levenscyclus, winterhardheid van plant en overlevingsstructuren, overlevings- en verspreidingsstructuren, secundaire dormantie, bestuivingswijze, aanwezigheid van kruisbare verwanten, zaadverstrooiing, en potentieel tot verwildering.

Ook werden inactivatiemethoden opgezocht en beschreven met speciale aandacht voor toepasbaarheid en hun voor- en nadelen. Ze werden ingedeeld volgens werkingsmechanisme (fysisch, biologisch, mechanisch of chemisch). Omdat slechts weinig referenties specifiek over ggo's handelen, werd ook gezocht, waar mogelijk, naar meer algemene onkruidbestrijdingstechnieken. Aan de andere kant kunnen onkruiden niet geheel worden vergeleken met cultuurgewassen, omwille van hun veerkracht. Mogelijk kunnen gg-gewassen daarom worden aangepakt met minder stringente methoden. Tot slot rapporteert de wetenschappelijke literatuur uiteenlopende cijfers over de effectiviteit van inactivatie, misschien als gevolg van verschillende milieuomstandigheden.

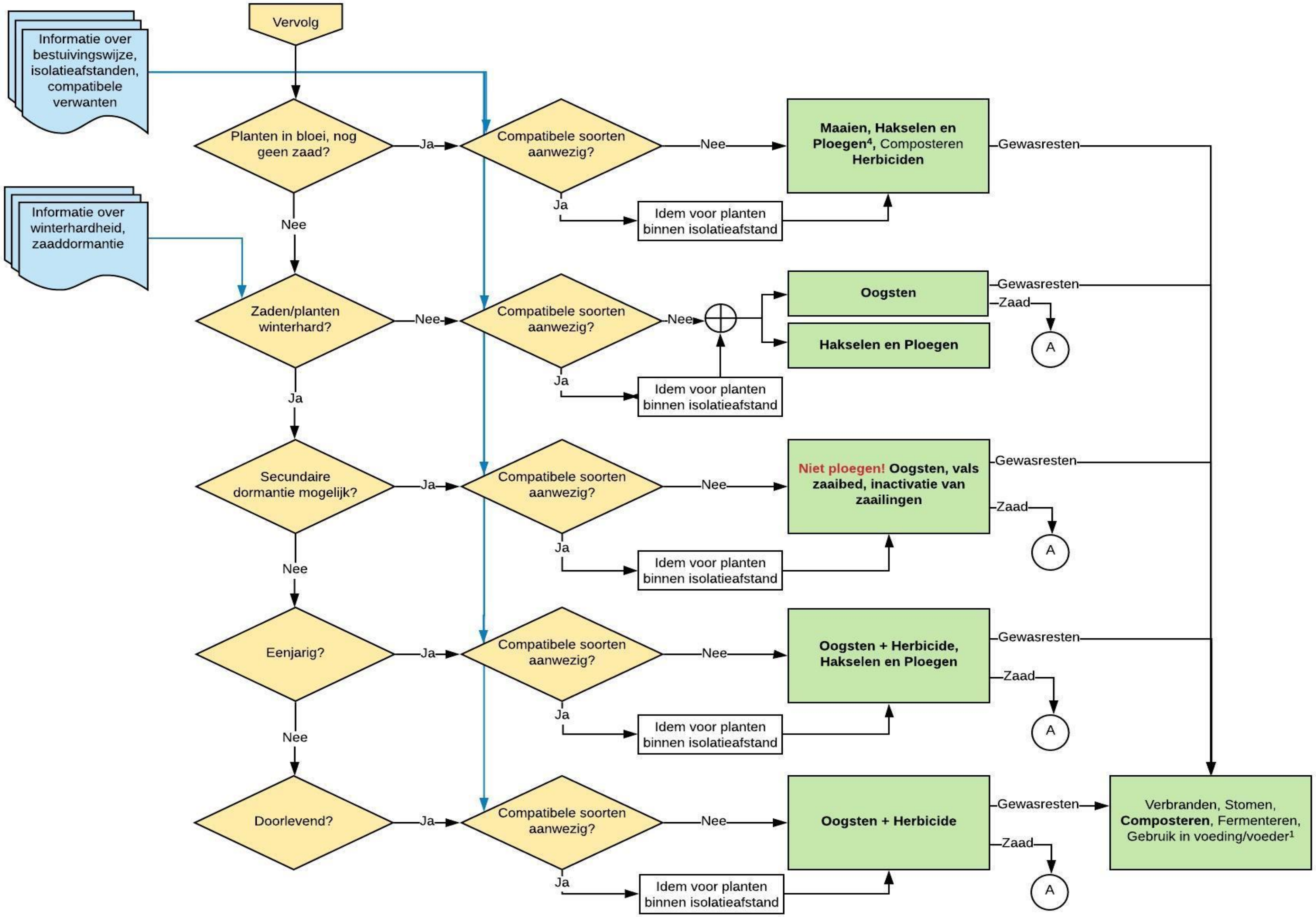
De geschiktheid van de diverse inactivatiemethoden voor de verschillende gg-plantencategorieën werd getoetst aan de kennis over de biologie van de planten en aan de toepasbaarheid en effectiviteit van de inactivatiemethoden. Er wordt een beslisboom (Figuur I) en een samenvattende tabel A voorgesteld die de meest geschikte methoden volgens type materiaal en/of ontwikkelingsstadium weergeven. Beiden zijn samenvattingen van de bevindingen en bieden een algemeen overzicht, maar de lezers worden uitgenodigd om de details na te kijken in de tabellen van Hoofdstuk 6.

Een bijkomende categorie omvat niet geclassificeerde soorten, zowel soorten die niet beschreven staan in een van de hogergenoemde categorieën, als soorten waarvoor tot dusver geen ggo's zijn geïntroduceerd in het milieu. Ze kunnen worden beoordeeld aan de hand van hun biologische eigenschappen (Hoofdstuk 2) en worden vergeleken met de groepering in Hoofdstuk 3. Als geen informatie voorhanden is of in dringende gevallen, kunnen de meest drastische inactivatiemethoden worden toegepast (bv. verbranding, herbicidebehandeling).

Figuur I – Samenvattende beslisboom om plantmateriaal te inactiveren naargelang het type materiaal en het ontwikkelingsstadium (blauwe vakjes duiden op informatie, geel – beslissingsvragen, groen – aanduiding van opties, meest praktische/effectieve oplossingen worden aangeduid in **vet**; compatibele species = seksueel compatibele species; ⊕ = opties)



Figuur I – vervolg



Tabel A – Samenvattende tabel van de meest geschikte inactivatiemogelijkheden per categorie en type van materiaal (voor zover relevant)

	Grassen	Winterharde graangewassen	Niet-winterharde graangewassen	kruisbloemigen en soorten met gelijkaardige karakteristieken	Eenjarige vlinderbloemigen en soorten met gelijkaardige karakteristieken	Doorlevende vlinderbloemigen	Knolgewassen	Wortelgewassen	Eenjarige fruit- & groetegewassesn (Niet-winterhard)	Bolgewassen	Pot- en bedding planten	Bomen
Zaad	S/ A / V	S/ A / V	S/ A / V	S/ A / V	S/ A / V	S/ A / V		S/ A / V	S/ A / V	S/ A / V	S/ A / V	S/ A / V
Gecoat zaad	V	V	V	V	V	V		V	V	V	V	
Knollen							Vr/ S					
Gezaaid, niet gekiemd	VoH	VoH	-	VoH	-	VoH	-	VoH	-	VoH		
Gezaaid, zaailingen en jonge planten in groeisubstraat				S/ A / V					S/ A / V	-		S/ A / V
Zaailingen en planten in rozetstadium				MeW / Ha/ Sch/ H					-	-		
Vegetatieve fase / schieterstadium (grondgebonden)	MeW / H	MeW / Sch +P / H	MeW / Sch +P / H	H	MeW / Ha / Sch / P / Ma/ H	MeW / H	H	MeW / H	Ma/ Sch / Ha +P	H	Rooien	Ha+
Vegetatieve + generatieve fase (niet grondgebonden / potten)									S/ A / V	S/ A / V	S/ A / V	
In bloei	Ma+H	Ha+P	Ha+P / O	O	Ha / Sch / P / Ma/ H	Ma+H/ H	O	Ui+ Dr	Ma/ Sch / Ha +P	H or S/ A / V	Rooien of S/ A/ V	
Zaadzetting	Ma+H	O+	Ha+P / O	O	Ha +P / O	Ma+H	O	Ui+V	Ma/ Sch / Ha +P	H or S/ A / V	Rooien of S/ A/ V	
Knol-/ wortel-/ bolstadium							O	H+ HaP		H or S/ A / V		
Zaadoogstverliezen	K+H/MeW	P / K+H/MeW	-	K+H/MeW	-	K+H/MeW	-	K+H/MeW	-	-		
Nazorg	Mon	-	-	Mon	-	Mon	Mon	Mon	-	Mon		

(Afkortingen: A = Autoclaveren; H = Herbicidebehandeling; H+HaP = Herbicidebehandeling gevolg door hakselen en ploegen; Ha = Hakselen; Ha+ = Hakselen + composteren of fermenteren of gebruik als houtsnippers; Ha+P = Hakselen en ploegen; K+H = laten kiemen + herbicidebehandeling; Vr = Bevriezen; O = Oogsten; O+ = Oogsten + behandeling (schijfploegen, ploegen of herbicide); V = Verbranden, Ma = Maaien; Ma+H = Maaien + Herbicide; MeW = Mechanisch wieden; Mon = Monitoren + inactivatie; S = Stomen; Sch = schijfploegen; Sch+P = schijfploegen en ploegen; VoH = Vooropkomstherbicide; P = Ploegen; Ui+Dr = Uittrekken en verdrogen; Ui+V= uittrekken en verbranden

Niettegenstaande de specifieke indicaties per categorie, kunnen de volgende conclusies getrokken worden:

- Met uitzondering van autoclaveren en verbranden, inactiveert geen van de methoden de planten en zaden voor 100%.
- Zaden van alle soorten kunnen op (bijna) dezelfde manier worden behandeld: verbranden is steeds geschikt, naast stomen en autoclaveren. Voor knolgewassen is bevriezing een bijkomende valabele optie.
- Natte biomassa kan altijd worden gecomposteerd of gefermenteerd, maar met aandacht voor mogelijk aanwezige zaden. In dat geval is het van belang de aangewezen temperatuur aan te houden gedurende een voldoend lange tijd. Stomen is ook een haalbare methode, vooral wanneer behalve biomassa nog ander materiaal aanwezig is (potten, grond, enz.).
- Eens op het veld is bespuiten met een geschikt herbicide om het gewas te doden de meest praktische optie. Bij de keuze van het herbicide moet rekening worden gehouden met het feit dat het ggo bestand kan gemaakt zijn tegen bepaalde herbiciden. Wanneer correct toegepast (ontwikkelingsstadium, dosis, weersomstandigheden), zal het resulteren in een hoge inactivatie-efficiëntie. Ook ander methoden zijn beschikbaar indien landbouwers ervoor kiezen geen chemische gewasbeschermingsmiddelen te gebruiken (landbouwers die pesticidegebruik willen reduceren, biologische landbouw). Mechanisch wieden ligt het meest voor de hand, naast het onderploegen van biomassa. Volgens de principes van de afvalhiërarchie (o.a. recycleren en nuttig gebruiken), zijn de opties maaien of oogsten en verzamelen van de zaden en/of gewasresten, de opties van composteren, fermenteren (biogas en ethanolproductie), biodieselproductie en aanwending als of in voeding en veevoeder in geval van vermenging met een toegelaten ggo, waardevolle alternatieven.
- Het onderwater zetten van akkers, grondstomen, solarisatie, mulchen en biologische grondontsmetting zijn ook mogelijk, maar kennen nadelen (bv. de lange inwerkingsperiode) en zullen mogelijk als onpraktisch worden gezien.
- Overwegende dat landbouwers waarschijnlijk zo snel mogelijk hun bedrijvigheden wensen te hervatten, zullen zij voor kortdurende behandelingen kiezen, indien mogelijk. Dit zal dikwijls neerkomen op het gebruik van herbiciden met een korte nawerking ofwel het onderploegen van plantenresten, temeer omdat zij over de geschikte machines beschikken. Met mulchen, anaerobe grondontsmetting, onderwater zetten, solarisatie kan mogelijk een groeiseizoen verloren gaan. De keuze van het volggewas bepaalt mede of monitoring en vernietigen van opslag mogelijk is. Dit kan leiden tot een aanpassing van de gebruikelijke vruchtwisseling.

In aanvulling op de technische aspecten van de inactivatietechnieken, kunnen er enkele algemene vaststellingen gedaan worden bouwend op de beperkte ervaring met eerdere incidenten en gesprekken met betrokken personen:

- De identificatie en kwantificatie van de ggo-vermenging zijn nodig voor een risicobeoordeling. Als het gaat over ggo's die in de EU zijn toegelaten voor invoer en gebruik in levensmiddelen en diervoeders, dan werden sommige milieurisico's[2] al geëvalueerd en kunnen andere toepassingen (zoals gebruik in voeding en/of veevoeder) toegelaten worden.
- Een belangrijke doelstelling is in te grijpen vooraleer de ggo's zich kunnen verspreiden in ruimte (via pollen of kruisbare verwanten) of in tijd (opbouw in de zaadbank). Voor gewassen die gezaaid worden kan dat laatste al het geval zijn bij het zaaien, omdat er altijd een kans is dat zaad in eerste instantie niet kiemt.
- De toepasbaarheid van sommige methoden hangt af van de beschikbaarheid van dure uitrusting en geschikte voorzieningen. Een autoclaaf kan gepast zijn voor de inactivatie van

[2] Niettegenstaande een milieurisicobeoordeling vereist is voor de toelating van invoer van levensvatbaar GG plantenmateriaal, het toepassingsgebied kan in meerdere opzichten verschillen: voor invoer zal er vooral aandacht worden besteed aan morsen tijdens transport, daar waar vermenging kan leiden tot aanwezigheid in de velden van de telers onder optimale teeltvoorwaarden. De relevantie van de evaluatie voor import moet dan ook geval-per-geval worden beoordeeld.

kleine hoeveelheden, maar minder geschikt voor grote volumina van met grond vervuild materiaal. Verder kunnen installaties van bv. biodiesel- of ethanolproductiesites gespecialiseerd zijn in het verwerken van bepaalde types uitgangsmateriaal. Mogelijk staan ze niet open voor een incidentele afwijkend partij. Vooral als de verwerking bijproducten oplevert bedoeld voor een andere sector (bv. veevoeder), moeten producten mogelijk gescheiden worden gehouden i.v.m. autorisatie en etiketteren van die producten.

- Idealiter wordt er zo dicht mogelijk bij de bron geïnactiveerd. Toch kan het zijn dat de meest efficiënte technieken transport vereisen, waarbij verlies van materiaal te allen tijde moet vermeden worden. De discrepantie tussen de regels van het ADR en de Regeling GGO in deze moeten worden uitgeklaard (verpakkingsinstructies).
- Monitoring kan helpen om de efficiëntie van de inactivatiemethoden te controleren of om resterende problemen op te sporen. Of het nuttig is, moet geval per geval worden bekeken afhankelijk van de plantensoort, het ontwikkelingsstadium, de mate van vermenging, gewasrotatie en of het ggo is toegelaten voor voedingsmiddelen, diervoeder en verwerking.
- Als de aanwezigheid van ggo's leidt tot verwijderen van het materiaal, kan er, bovenop het aanwijzen van de geschikte methoden, duidelijker worden aangegeven wie verantwoordelijk is (waaronder inspectie) en welke verificatie en rapportering er wordt verwacht. Een gedocumenteerd proces en opvolging kunnen nuttig zijn voor het aantonen van naleving en om meer te weten te komen over goede praktijken.

Ondanks de wereldwijde schaal waarop ggo's worden gebruikt, blijft het aantal incidenten dat wordt gerapporteerd en dat inactivatie van plantmateriaal noodzaakt, beperkt. Echter, wanneer meer landen toepassingen in de plantenbiotechnologie introduceren en producttoelating niet gesynchroniseerd wordt, zal de situatie er niet op vooruit gaan. Bovendien, eens een niet-toegelaten ggo is ontdekt, kan de impact verreikend zijn, wat snelle actie vereist. Naarmate technieken worden ontwikkeld, kunnen meer geschikte mogelijkheden beschikbaar komen. De benadering zoals voorgesteld in deze studie, waarbij de keuze van de inactivatiemethode wordt verantwoord op basis van biologische eigenschappen van de gg-plantensoort, blijft overeind voor toekomstige incidenten.

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Abbreviations

ADR	*Accord européen relatif au transport international des marchandises Dangereuses par Route*
CABI	Centre for Agriculture and Bioscience International
CFIA	Canadian Food Inspection Agency
COGEM	Netherlands Commission on Genetic Modification *Commissie Genetische Modificatie*
CTGB	Board for the Authorisation of Plant Protection Products *College voor de toelating van gewasbeschermingsmiddelen en biociden*
DDGS	Dried Distillers' Grains with Solubles
DNA	Deoxyribonucleic acid
EU	European Union
GEAC	Genetic Engineering Appraisal Committee, Ministry of Environment, Forest and Climate Change, India
ggo	Genetisch gemodificeerd organisme
GM	Genetically modified
GMO	Genetically modified organism
ILT	Ministry of Infrastructure and Water Management - Human Environment and Transport Directorate *Ministerie van Infrastructuur en Waterstaat - Inspectie Leefomgeving en Transport*
ISAAA	International Service for the Acquisition of Agri-biotech Applications
ISF	International Seed Federation
NVWA	Netherlands Food and Consumer Product Safety Authority *Nederlandse Voedsel- en Warenautoriteit*
OECD	Organisation for Economic Co-operation and Development
OGTR	Office of the Gene Technology Regulator, Australia
SBB	Belgian Biosafety Server
VLG	*Regeling vervoer over land van gevaarlijke stoffen*

Introduction

Genetically modified crops are grown and traded in the world for nearly 25 years. In many jurisdictions an authorisation is needed before the GM crop can be placed on the market, for cultivation, use as feed or food and other applications. Due to asynchronous authorisations between countries international trade is experiencing difficulties (Stein and Rodriguez-Cerezo, 2009). The inadvertent presence of non-authorised GM events in commodity shipments and seed lots relative to the importing country has its impact on trade. Adventitious presence of non-authorised GM events not only originates from events deregulated elsewhere in the world, but also from inadvertent commingling of GM material during research and development (Bashandy and Teeri, 2017) and field trials[3]. Commingling may occur between the same or different species.

In the EU Directive 2009/41/EC[4] regulates GMOs in contained use, Directive 2001/18/EC[5] and Regulation (EC) No 1829/2003[6] regulate respectively the deliberate release in the environment of GMOs and GM food and feed. Only one GM event, MON810 in maize, is authorised for cultivation in the EU according to Directive 2001/18/EC. Moreover, many GM commodities are imported into the EU following an approval according to Regulation (EC) No 1829/2003.

In the Netherlands the Ministry of Infrastructure and Water Management, Human Environment and Transport Directorate (ILT) is responsible for monitoring imported plants and seeds for sowing for unauthorised presence of GM events, amongst others. Also, companies, organisations and other authorities may notify the ILT of the presence of unauthorised GMOs on Dutch territory. Table 1 presents an overview of recent incidents in the Netherlands. On three occasions COGEM was asked to advise on the risk for the environment and, where appropriate, on proper inactivation techniques. The maize seed lot containing trace amounts of the MON810 event was not inactivated as the event is authorised for cultivation in the EU.

Table 1. Cases of inadvertent mixing of unauthorised GM seeds and plants in the Netherlands since 2012 (source ILT)

Crop	Commingling event	Concentration	COGEM advice	Type of material
Cotton	MON531	<0,1%	(COGEM, 2015)	Seed
Oilseed rape (for oil production)	RT73	<0,1%	No	Grain
Maize	MON89034	<0,1%	No	Seed
Maize	MON810	<0,1%	No	Seed
Soya bean	PR91M10	<0,1%	No	Plants (field trial)
Petunia	-	-	(COGEM, 2017)	Pot plants
Oilseed rape (seed for sowing)	GT73	<0,1%	(COGEM, 2019)	Plants (field)

Once detected the material that is destined for sowing or planting or that is already in the field needs to be inactivated. Several inactivation methods are available, such as composting, ploughing, herbicide spraying, incineration. Depending on the species and growing stage one or the other method is more suitable. In order to have a ready-to-use guideline, this report inventories and classifies inactivation methods in relation to the plant species and development stage.

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[3] E.g. Glyphosate-Resistant Wheat Incidents: https://www.aphis.usda.gov/wcm/connect/aphis_content_library/sa_our_focus/biotechnology/hot_topics/glyphosate_resistant_wheat/wheat_investigation

[4] Directive 2009/41/EC of the European Parliament and of the Council of 6 May 2009 on the contained use of genetically modified micro-organisms. Official Journal L 125, 21/05/2009, p.75-97.

[5] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. Official Journal L 106, 17/04/2001, p.1-39.

[6] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. Official Journal L 268, 18/10/2003, p.1-23.

1 Purpose of the study

This study was set up to develop guidelines for the inactivation of plants and seeds that unintentionally became commingled with non-authorised GM events. The study is restricted to seeds for sowing and plants destined to or already present in the field, garden or greenhouse. Grain and plants destined to be used in food or feed are out of the scope. Nevertheless, some of the proposed methods may be equaly suitable to inactivate food and feed lots.

This study was expected to result in een overview of methods suitable for inactivation of plant material, their efficacy, and a description of the situations and plant types where these methods may be applied. The research approach was structured as follows:

1. Identify plant species that may require inactivation due to the presence of unauthorised GMOs;
2. Identify inactivation methods and describe advantages and shortcomings;
3. Determine which inactivation method is suitable for which plant species and for which developmental stage and its efficacy to inactivate.

2 Methods

2.1 Inventory of the relevant plant species

The first source of GM plant species that unintentionally might end up in commercial circuit in the Netherlands are those that are commercially available elsewhere in the world, but not authorised for cultivation in the EU. Today, only one event is approved for cultivation in the EU, i.e. MON810 (unique identifier MON-ØØ81Ø-6). For food, feed and other uses 108 events in 8 plant species were granted authorisation in the EU since 1994[7]. Authorisation is granted for a period of 10 years and has to be renewed after this period. For some of them applicants did not file an application for renewal and the authorisation period was not extended.

A second source are GM plants that are worldwide included in R&D field trials. Indeed, several incidents concern GM events that were investigated and trialled in field trials in the developmental phase, but were not advanced or did not yet obtain commercial approval. Species that are under investigation in research facilities only, were not considered.

The potential plant species were retrieved form publicly available data on field trials[8] and data of commercial approvals worldwide[9]. Perseus maintains a GM field trial database that is composed using data that are publicly available, either from regulatory authorities, companies' websites, newsletters etc. The database contains data from the past 20 years. The early period of plant genetic modification is not included, but since most species trialled in that period have been continued, the database provides an almost full coverage. The International Service for the Acquisition of Agri-biotech Applications (ISAAA) collects data on GM crop events that have been approved for commercialisation (cultivation, food and feed). The list is based on decision documents of each approving country, the Biosafety Clearing House of the Convention on Biological Diversity, and peer-reviewed scholarly articles.

From this list a selection was made based on the likelihood of being imported in the Netherlands as seeds for sowing, as pot plants or as vegetative reproduction tissue. Also, trees and bushes that are

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[7] https://ec.europa.eu/food/plant/gmo/eu_register_en and http://www.isaaa.org/

[8] Perseus' field trial database; GM trials are only included as far as they contain plants that are regarded as GM in the different jurisdictions.

[9] http://www.isaaa.org/

able to grow in the Netherlands outdoor or indoors were retained. Some minor crops and herbs were not considered because of their minor importance.

2.2 Inventory of inactivation methods

Potential methods of inactivation are mentioned in regulations, field trial authorisations, COGEM advices, biosafety guidelines, weed management guidelines, guidelines for the inactivation of quarantine organisms, earlier incidents, literature and obtained through personal contacts.

Web of Science, Scopus and Google Scholar were searched using appropriate search criteria.
For all methods a description is included of their mode of action, the inactivation efficacy, the advantages and disadvantages.

2.3 Suitability of inactivation methods

The relevant plant species were classified according to their biological characteristics and the prevalence of the species and compatible relatives in the Netherlands. These are:

- Type of life cycle: annual, biennial, perennial,
- Winterhardiness of the plant,
- Survival/dissemination structure,
- Winterhardiness of the survival structure,
- Secondary seed dormancy,
- Pollination method and presence of compatible species,
- Seed shatter,
- Potential to form feral populations.

Comparing the characteristics of an inactivation method with the characteristics of the non-authorised plants will result in a ranking of suitability. This assessment approach eventually supports the forecast of the potential for survival when applying a certain inactivation technique.

2.4 How to use this report?

In Chapter 6 possible inactivation methods are listed for each plant group and development stage. The most suitable methods are indicated in bold.

Once the commingling plant species is identified in an incident, there are 2 situations:

- The species is listed in Table 4.
  The corresponding inactivation suitable for this species can be found in Chapter 6 in the section of the relevant group.
- The species is not listed in Table 4.
  Information on the biological features listed in 2.3 should be looked up. This information can be compared with the characteristics of the plant groups of Table 4. The corresponding inactivation suitable for this species can be found in Chapter 6 in the section of the most corresponding group.

Note: whenever an inactivation method is selected for a specific case, the applicable legislation should be verified. Legislation may evolve and/or may impose local restrictions, that will impact the choice of the options presented in this report.

3 Plant species

3.1 Relevant plant species

Thirty-two GM species have a commercial approval in at least one country. They are listed in Table 2.

Table 2: List of commercially approved GM crop species (source ISAAA)

Scientific name	Crop name	Scientific name	Crop name
Agrostis stolonifera	Creeping Bent grass	*Malus domestica*	Apple
*Ananas comosus**	Pineapple	*Medicago sativa*	Alfalfa, lucerne
Beta vulgaris	Sugar beet	*Nicotiana tabacum*	Tobacco
Brassica napus	Argentine canola	*Oryza sativa*	Rice
Brassica rapa	Polish canola	*Petunia* x *hybrida*	Petunia
Capsicum annuum	Sweet pepper	*Phaseolus vulgaris*	Bean
Carica papaya	Papaya	*Populus* sp.	Poplar
Carthamus tinctorius	Safflower	*Prunus domestica*	Plum
Cichorium intybus	Chicory	*Rosa hybrida*	Rose
Cucumis melo	Melon	*Saccharum* sp.	Sugarcane
Cucurbita pepo	Squash	*Solanum lycopersicum*	Tomato
Dianthus caryophyllus	Carnation	*Solanum melongena*	Eggplant
Eucalyptus sp.	Eucalyptus	*Solanum tuberosum*	Potato
Glycine max	Soya bean	*Triticum vulgare**	Wheat
Gossypium hirsutum	Cotton	*Vigna unguiculata*	Cowpea
Linum usitatissimum	Linseed/flax	*Zea mays*	Maize

* not approved for cultivation

In the Perseus field trial database about 160 different plant species are identified (Table 3). These include the 'big' crops, such as maize, soya bean, cotton and oilseed rape, next to other arable, vegetable and ornamental crops. Tree species like eucalyptus and poplar, as well as fruit trees, apple, plum, pear and papaya are being trialled as well.

Table 3: List of GM crop species that have been or are being trialled in the field (source Perseus field trial database)

Scientific name	Common name	Family name
Abelmoschus esculentus	Okra	Malvaceae
Agrostis canina	Velvet bent grass	Poaceae
Agrostis sp.	Bent grass, bent	Poaceae
Agrostis stolonifera (Agrostis palustris)	Creeping bent grass	Poaceae
Allium cepa	Onion	Amaryllidaceae
Ananas comosus	Pineapple	Bromeliaceae
Anthurium andreanum	Anthurium	Araceae
Anthurium sp.	Anthurium	Araceae
Arabidopsis thaliana	Thale cress, mouse-ear cress	Brassicaceae
Arachis hypogaea	Peanut, groundnut	Fabaceae
Artemisia annua	Sweet wormwood	Asteraceae
Atropa belladonna	Belladonna, deadly nightshade	Solanaceae
Begonia semperflorens	Begonia	Begoniaceae

Scientific name	Common name	Family name
Beta vulgaris	Sugar beet, fodder beet	Amaranthaceae
Betula pendula	Birch	Betulaceae
Brassica carinata	Ethiopian mustard	Brassicaceae
Brassica juncea	Brown mustard, Indian mustard	Brassicaceae
Brassica napus	Oilseed rape, Argentine canola	Brassicaceae
Brassica oleracea	Cabbage, vegetable brassicas	Brassicaceae
Brassica rapa	Chinese cabbage, Polish canola	Brassicaceae
Cajanus cajan	Pigeon pea	Fabaceae
Camelina sativa	Gold-of-pleasure, false flax	Brassicaceae
Capsicum annuum	Pepper, chili pepper, bell pepper	Solanaceae
Carica papaya	Papaya	Caricaceae
*Carthamus tinctorius**	Safflower	Asteraceae
Castanea dentata	American chestnut	Fagaceae
Chrysanthemum × morifolium	Chrysanthemum, chrysanths	Asteraceae
Cicer arietinum	Chickpea	Fabaceae
*Cichorium intybus**	Chicory, Belgian endive	Asteraceae
Citrullus lanatus var. *lanatus*	Watermelon	Cucurbitaceae
Citrus × aurantiifolia	Mexican lime, Key lime	Rutaceae
Citrus × aurantium	Bitter orange	Rutaceae
Citrus × paradisi	Grapefruit	Rutaceae
Citrus × paradisi × Poncirus trifoliata	Citrumelo, citromelo	Rutaceae
Citrus limon	Lemon	Rutaceae
Citrus reticulata × Poncirus trifoliata	Citrandarin	Rutaceae
Citrus sinensis	Orange tree	Rutaceae
Citrus sp.	Citrus	Rutaceae
Citrus sinensis x Poncirus trifoliata	Citrange	Rutaceae
Cornus canadensis	Bunchberry	Cornaceae
Crambe abyssinica	Crambe	Brassicaceae
Crambe sp.	Crambe	Brassicaceae
Cryptomeria japonica	Japanese cedar	Cupressaceae
Cucumis melo	Muskmelon, Melon	Cucurbitaceae
Cucumis sativus	Cucumber, gherkin	Cucurbitaceae
Cucurbita sp.	Squash, pumpkin	Cucurbitaceae
Cuphea viscosissima x Cuphea lanceolata f. silenoides	Cuphea	Lythraceae
Cyclamen persicum	cyclamen	Primulaceae
Cynodon dactylon	Bermudagrass	Poaceae
*Daucus carota**	carrot	Apiaceae
Dendrobium sp.	Dendrobium orchid	Orchidaceae
Dianthus caryophyllus	Carnation	Caryophyllaceae
Diospyros sp.	Persimmon	Ebenaceae
Elaeis guineensis & Elaeis oleifera	Oil palm	Arecaceae
Eucalyptus camaldulensis	River red gum	Myrtaceae
Eucalyptus grandis	Flooded gum, rose gum	Myrtaceae
Eucalyptus sp.	Eucalypt	Myrtaceae
Eucalyptus urophylla	Timor white gum	Myrtaceae
Festuca arundinacea	Tall fescue	Poaceae
Fragaria x ananassa	Strawberry	Rosaceae
Gladiolus sp.	Gladiolus	Iridaceae
Glycine max	Soya bean	Fabaceae
Gossypium arboreum	Tree cotton	Malvaceae
Gossypium hirsutum	Cotton	Malvaceae
*Gypsophila paniculata**	Baby's breath	Caryophyllaceae
*Helianthus annuus**	Sunflower	Asteraceae
Hevea brasiliensis	Rubber tree	Euphorbiaceae
Hordeum vulgare	Barley	Poaceae

Scientific name	Common name	Family name
Ipomoea × sloteri	Cardinal climber (cypress vine)	Convolvulaceae
Ipomoea batatas	Sweet potato	Convolvulaceae
Iris sp.	Iris	Iridaceae
Jatropha curcas	Jatropha	Euphorbiaceae
Juglans sp.	Walnut	Juglandaceae
Lactuca sativa	Lettuce	Asteraceae
Lactuca sativa L. var. longifolia	Romaine lettuce	Asteraceae
Lens culinaris	Lentils	Fabaceae
Lepidium campestre	Field cress, field pepperwort, field pepper weed	Brassicaceae
Lilium longiflorum	Easter lily	Liliaceae
*Linum usitatissimum**	Flax, Linseed	Linaceae
Liquidambar sp.	Sweetgum	Altingiaceae
Lolium multiflorum (now *Festuca perennis*)*	Italian ryegrass	Poaceae
Lolium perenne	Perennial ryegrass	Poaceae
*Lupinus angustifolius**	Narrow-leafed lupin	Fabaceae
Malus domestica	Apple tree	Rosaceae
Manihot esculenta	Cassava, yucca	Euphorbiaceae
*Medicago sativa**	Alfalfa, lucerne	Fabaceae
Medicago truncatula	Barrel clover	Fabaceae
Mentha × piperita	Peppermint	Lamiaceae
Miscanthus sp.	Silver grass, elephant grass	Poaceae
Musa acuminata	Cavendish banana	Musaceae
Musa acuminata x Musa balbisiana	Banana	Musaceae
Musa sp.	Banana	Musaceae
Nicotiana attenuata	Coyote tobacco	Solanaceae
Nicotiana glauca	Tree tobacco	Solanaceae
Nicotiana sylvestris	Wild tobacco	Solanaceae
Nicotiana tabacum	Tobacco	Solanaceae
Nicotiana tabacum x Nicotiana glauca	Nicotiana hybrid	Solanaceae
Olea europaea	Olive tree	Oleaceae
Ornithogalum x thyrsoides	Chincherinchee	Asparagaceae
Oryza sativa	Rice	Poaceae
Panicum virgatum	Switchgrass	Poaceae
Papaver somniferum	Breadseed poppy	Papaveraceae
Parthenium argentatum	Guayule	Asteraceae
Paspalum notatum	Bahia grass	Poaceae
Pelargonium sp.	Geranium, pelargonium	Geraniaceae
Persea americana	Avocado	Lauraceae
Petunia x hybrida	Petunia	Solanaceae
Phalaenopsis amabilis	Phalaenopsis, moon orchid	Orchidaceae
Phalaris canariensis	Canary seed, Canary grass	Poaceae
Phaseolus vulgaris	Common bean, pinto bean	Fabaceae
Physalis peruviana	Cape gooseberry	Solanaceae
Picea spp.	White spruce and black spruce	Pinaceae
Pinus radiata	Radiata pine, Monterey pine	Pinaceae
Pinus rigida x Pinus taeda	Pitch pine x loblolly pine, loblolly hybrid	Pinaceae
Pinus taeda	Loblolly pine	Pinaceae
Pisum sativum	Pea	Fabaceae
*Poa pratensis**	Kentucky bluegrass, smooth meadow-grass	Poaceae
Poa pratensis x Poa arachnifera	Heat-tolerant bluegrass	Poaceae
Poncirus trifoliata	Trifoliate orange	Rutaceae
Populus alba	White poplar, silver poplar	Salicaceae

Scientific name	Common name	Family name
Populus alba x Populus tremula	Grey poplar	Salicaceae
Populus deltoides	Eastern cottonwood	Salicaceae
Populus deltoides x Populus nigra	Canadian poplar	Salicaceae
Populus spp.	Poplar	Salicaceae
Populus tremula x Populus alba	Grey poplar	Salicaceae
Populus tremula x Populus tremuloides	Hybrid aspen	Salicaceae
Populus trichocarpa	Black cottonwood	Salicaceae
Populus x canescens	Grey poplar	Salicaceae
Populus x *canadensis* (*Populus* x *euramericana*)	Canadian poplar	Salicaceae
Prunus domestica	Plum tree	Rosaceae
Psathyrostachys juncea	Russian wildrye	Poaceae
Pyrus communis	Pear	Rosaceae
Ricinus communis	Castor bean	Euphorbiaceae
Rhododendron sp.	Rhododendron	Ericaceae
Rosa hybrida	Rose	Rosaceae
Rosa sp.	Rose	Rosaceae
Rubus idaeus	Red raspberry	Rosaceae
Saccharum officinarum	Sugarcane	Poaceae
Saccharum officinarum x *Saccharum spontaneum*	Sugarcane	Poaceae
Saccharum spp.	Sugarcane	Poaceae
Salvia sclarea	Clary, Clary sage	Lamiaceae
Solanum lycopersicum	Tomato	Solanaceae
Solanum melongena	Aubergine, Eggplant, Brinjal	Solanaceae
Solanum nigrum	Black nightshade	Solanaceae
Solanum tuberosum	Potato	Solanaceae
Sorghum bicolor	Sorghum, Sweet Sorghum	Poaceae
Sorghum sp.	Sorghum	Poaceae
Stenotaphrum secundatum	St. Augustine grass, buffalo grass	Poaceae
Tagetes spp.	Marigold	Asteraceae
*Trifolium repens**	White Clover	Fabaceae
x Triticosecale rimpaui Wittmack	Triticale	Poaceae
*Triticum aestivum**	Wheat	Poaceae
Triticum monococcum	Einkorn wheat	Poaceae
Triticum turgidum ssp. durum	Durum wheat	Poaceae
Ulmus americana	American elm	Ulmaceae
Vaccinium sp.	Blueberry	Ericaceae
Vigna unguiculata	Cowpea, black-eyed pea	Fabaceae
Vitis rotundifolia	Muscadine grapevine	Vitaceae
Vitis vinifera	Common grapevine	Vitaceae
Vitis vinifera x Vitis berlandieri	Grapevine hybrid (rootstock)	Vitaceae
Zantedeschia sp.	Calla	Araceae
Zea mays	Maize	Poaceae

In black: prevalent in agriculture and horticulture, gardens, floral borders, indoors and in "the wild" in the Netherlands (part of the information from https://www.nederlandsesoorten.nl/ and http://www.soortenbank.nl/); In blue: all other species
* species that, next to being cultivated as a crop, may also be present in seed mixes for field edges (De Win and Vervaeke, 2015; Temmerman et al., 2012) and in general flower seed mixes for sale[10]

From the combined list of species, the most relevant species were selected. The first selection criterium was the prevalence in the Netherlands (in black in Table 3). Next, the emphasis was put on species that are generally imported as seeds. Nevertheless, species that are multiplied vegetatively such as potatoes, fruit trees and ornamentals are also included, as well as species that are occasionally planted on a small scale and in private gardens. Also, common pot and bedding plants

[10] e.g. https://medigran.nl/mengsel-overzicht/

were included. Popular flower seed mixes, were also checked for the presence of species listed in Table 3. Minor crops and herbs were not retained.

3.2 Classification of relevant plant species

To select for a suitable and effective inactivation method, an assessment based on biological characteristics of the plant species has to be performed. To that end biology documents, scientific literature and reports from scientific institutes were consulted (see 8.1). The characteristics relevant for the purpose of this report are listed in 2.3. The results of this exercise are presented in Table 4. Species with similar features are grouped for simplification, with one or two type species. Although often species in the same species family tend to be grouped together, this is not always the case (e.g. legumes). Vice versa, species from different families may have similar characteristics (e.g. cotton and sunflower together with annual legumes).

In this table species from the same genus are taken together, although they may differ in some aspects. E.g., in the *Citrus* genus some winter hardy species are present (*Citrus* × *paradisi* × *Poncirus trifoliata*, *Citrus reticulata* × *Poncirus trifoliata* and *Citrus sinensis x Poncirus trifoliata*), while others are cold-sensitive.

Table 4: Species groups and their biological characteristics

Species	Common name	Annual/ Perennial[1]	Winterhardiness plant	Survival/ dissemination structure	Winterhardiness survival structure	Secondary seed dormancy[1]	Pollination[2]	Compatible species in the Netherlands	Seed shatter[1]	Feral populations in the Netherlands
Grasses										
Agrostis sp.	Bent grass	Perennial	Yes	Seed, rhizomes, stolons, tillers	Yes	No	Cross, wind	Yes	Yes	Yes
Cynodon dactylon	Bermudagrass									
Festuca arundinacea	Tall fescue									
Lolium perenne	Perennial ryegrass									
Poa pratensis	Kentucky bluegrass									
Miscanthus sp.	Silver grass			*M. sinensis* (2n): seed, rhizomes *M. × giganteus* (3n): rhizomes						No
Lolium multiflorum	Italian ryegrass	Annual, biennial		Seed		No	Cross, wind	Yes	Yes	Yes
Cereals (winter hardy)										
Triticum spp.	**Wheat**	Annual	Spring and winter varieties	Seed, tillers	Yes	No	Self (cross, wind)	(Crop)	No	No
Hordeum vulgare	Barley									
x Triticosecale rimpaui Wittmack	Triticale		Yes							
Phalaris canariensis	Canary seed					Yes				
Cereals (non-winter hardy)										
Zea mays	**Maize**	Annual	No	Seed, tillers	No	No	Cross, wind	Crop	No	No
Sorghum bicolor	Sorghum									
Oryza sativa	Rice									
Crucifers and species with similar characteristics										
Brassica sp.	**Oilseed rape**, cabbage	Annual (biennial)	Yes/no	Seed	Yes	Yes	Self and cross, wind and insects	Crop, wild crucifers	Yes	Yes
Arabidopsis thaliana	Arabidopsis									
Camelina sativa	False flax									
Lepidium campestre	Field cress									
Crambe sp.	Crambe								No	
Carthamus tinctorius	Safflower	Annual	Rosette: yes Stem: no	Seed	Yes	No	Self and cross, wind and insects	Crop	No	Yes
Linum usitatissimum	Linseed/flax		No			Yes	Self	(Crop)	Yes	
Legumes (annual) and species with similar characteristics										
Glycine max	**Soya bean**	Annual	No	Seed	No	No	Self	(Crop)	No	No
Cicer arietinum	Chickpea									

Species	Common name	Annual/ Perennial(1)	Winterhardiness plant	Survival/ dissemination structure	Winterhardiness survival structure	Secondary seed dormancy(1)	Pollination(2)	Compatible species in the Netherlands	Seed shatter(1)	Feral populations in the Netherlands
Lens culinaris	Lentil									
Medicago truncatula	Barrel clover									
Phaseolus vulgaris	Common bean									
Pisum sativum	Pea									
Lupinus angustifolius	Lupin		Yes		Yes					
Gossypium sp.	**Cotton**	Annual	No	Seed	No	No	Self	-	No	No
Helianthus annuus	Sunflower						Self/cross, insects	Crop	by birds	
Legumes (perennial)										
Medicago sativa	**Alfalfa,** lucerne	Perennial	Yes	Seed, stolons	Yes	Yes	Cross, insects	Crop Wild relatives	Yes	Yes
Trifolium repens	White clover									
Tuber crops										
Solanum tuberosum	**Potato**	Annual	No	Tubers, seed	Tuber: no Seed: yes	Yes	Self (cross, wind)	Crop	No	No
Ipomoea batatas	Sweet potato						Cross, insects			
Root crops										
Beta vulgaris	**Sugar beet**	Annual (biennial)	No	Seed (crown, root cuttings)	Yes	Yes	2n: cross, wind (insects) 3n: sterile	Crop and wild relatives	(Yes)	No(3)
Cichorium intybus	Chicory						Cross, insects			
Daucus carota	Carrot			Seed		Yes?				
Other annual fruit & vegetable species (non-winter hardy)										
Capsicum annuum	Pepper	Annual	No	Seed	No	No	Self/cross, insects	Crop	No	No
Citrullus lanatus var. lanatus	Watermelon									
Cucumis melo	Melon									
Cucurbita sp.	Pumpkin, squash									
Nicotiana sp.	Tobacco									
Physalis peruviana	Cape gooseberry									
Solanum lycopersicum	Tomato									
Solanum melongena	Aubergine, eggplant, brinjal									
Cucumis sativus	Cucumber, gherkin						Parthenocarpy			
Lactuca sativa	Lettuce				Yes		Self	(Crop and wild relative)		Yes

Species	Common name	Annual/ Perennial[(1)]	Winterhardiness plant	Survival/ dissemination structure	Winterhardiness survival structure	Secondary seed dormancy[(1)]	Pollination[(2)]	Compatible species in the Netherlands	Seed shatter[(1)]	Feral populations in the Netherlands
Bulbs (winter hardy)										
Iris sp.	Iris	Perennial	No (above ground)	Bulbs, rhizomes and seed	Yes	Yes?	Self/cross, insects	Yes	No	No
Lilium sp.	Lily			Bulbs, bulbils, scales and seed		?				
Bulbs (non-winter hardy)										
Allium cepa	**Onion**	Annual (biennial)	No	Bulbs, bulbils and seeds	Seed: yes Bulbs: no	No	Self/cross, insects	Crops	No	No
Ornithogalum	Chincherinchee	Perennial	Yes/no			?	Cross, insects			
Gladiolus sp.	Gladiolus		No	Corms, cormels and seeds	Seed: yes Corms: no					
Pot and bedding plants										
Anthurium	Anthurium	Annual	No	Seed	No	No	Cross, insects	Same species	No	No
Begonia semperflorens	Begonia									
Petunia x hybrida	Petunia					Yes				
Tagetes sp.	Marigold					?				
Cyclamen persicum	Cyclamen	Perennial		Seed and tuber						
Dendrobium sp.	Dendrobium orchid			Seed						
Pelargonium sp.	Pelargonium			Seed, cuttings						
Phalaenopsis amabilis	Phalaenopsis			Seed						
Zantedeschia sp.	Calla									
Chrysanthemum × *morifolium*	Chrysanths		Yes/no	(Seed)[(4)]						
Dianthus caryophyllus	Carnation		Yes	Seed	Yes					
Gypsophila paniculata	Baby's breath		Yes	Seed, rhizome		No	Self/cross, insects		Disperse seeds as tumbleweed	Yes
Trees (winter hardy)										
Betula pendula	Birch	Tree	Yes	Seed/grafts/ cuttings	Yes	?	Cross Wind/insects	Yes	Yes	Yes
Cornus canadensis	Bunchberry									No
Cryptomeria japonica	Japanese cedar									
Diospyros sp.	Persimmon									
Juglans sp.	Walnut									
Liquidambar sp.	Sweetgum									
Malus domestica	Apple tree									
Picea spp.	Spruce									

Species	Common name	Annual/ Perennial[(1)]	Winterhardiness plant	Survival/ dissemination structure	Winterhardiness survival structure	Secondary seed dormancy[(1)]	Pollination[(2)]	Compatible species in the Netherlands	Seed shatter[(1)]	Feral populations in the Netherlands
Populus spp.	Poplar									
Prunus domestica	Plum tree									
Pyrus communis	Pear									
Rhododendron sp.	Rhododendron	Bush								Yes
Rosa sp.	Rose									No
Rubus idaeus	Red raspberry									
Vaccinium sp.	Blueberry									
Vitis vinifera	Grapevine									
Trees (non-winter hardy)										
Citrus sp.	Citrus	Tree	Yes/no	Seed/grafts/ cuttings	?	?	Cross	No	Yes	No
Musa sp.	Banana	Perennial (pseudostem)	No	Suckers (seed)	No	-	-	No	No	No

(1) Characteristic as a crop
(2) The predominant way of pollination is indicated
(3) Due to the biennial character, the crop is harvested before flowering, although some bolters may arise in the first year
(4) Due to late flowering outdoors, seeds are not relevant

4 General observations

4.1 Experience

Although measures are in place to prevent commingling of unauthorised GM plant material, accidental mixing can nevertheless occur. Seed and plant imports are selectively tested for the presence of non-authorised GM events. Still, an incident of commingling may be noticed later on in any stage of the life cycle of a plant. Also, because of the time testing requires, the material may already be advanced in the chain. This means that plants may already be sown in the field or even flower and have set seed (Figure 1). Inactivation methods need to be adapted to these circumstances.

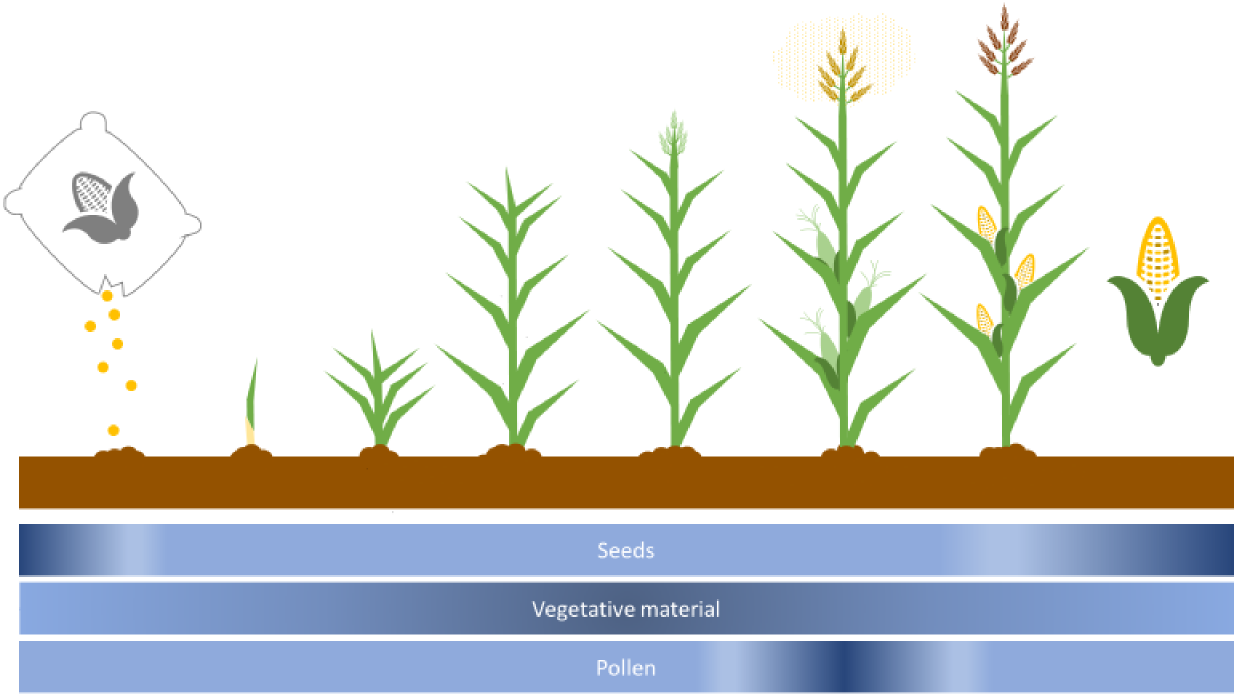

Figure 1 Plant developmental stages at which the incident may be noticed

In this context inactivation method means a method that devitalises the plant material, i.e. the material is no longer capable of growing, disseminating or multiplying. The method is not required to break down DNA.

Several methods are already described in relation to field trials with GM plants. E.g. in the field trial authorisation documents[11] for the Netherlands, the general requirements mention several possibilities for waste treatment[12]. Waste originating from field trials can either be:
a. killed and subsequently incorporated into the soil or burnt on the field;
b. destructed by autoclaving;
c. transported to an incineration facility;
d. processed according to the suggested method in the application form.

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[11] Bureau GGO: vergunningendatabase biotechnologie, https://www.ggo-vergunningverlening-zoeken.nl/

[12] 1. Van het proefobject afkomstig afval van genetisch gemodificeerde planten wordt op één van de onderstaande wijzen verwerkt:
a. doden en vervolgens onderwerken of verbranden op het proefobject;
b. vernietigen door middel van autoclaveren;
c. afvoeren naar een inrichting voor de verbranding van bedrijfsafvalstoffen en daar ter onmiddellijke verbranding aanbieden.
d. conform punt E.20, pagina xx van de aanvraag.

The Dutch GMO legislation (*Regeling GGO*[13]) mentions in Annex 10, Article A:8 the same inactivation methods for potatoes genetically modified with a reduced amylose content.

Protocols for field releases with transgenic plants[14] from the Belgian Biosafety Server (SBB[15]) list:

- incineration or heat treatment for left-over and waste seed; in case of seeds treated with fungicides or insecticides: inactivation as is customary in the seed industry;
- heat treatment (*e.g.* autoclave), incineration or desiccation on the spot for discarded plants;
- herbicide (systemic non-selective) spraying and/or chopping and ploughing for plants that are no longer needed (*e.g.* male plants in seed production);
- incineration and ploughing of plants;
- leaves and sugar beet and chicory crowns: fine chopping and spreading on the field;
- harvested and chopped/grated sugar beets and chicory roots: landfill, incineration, ploughing, composting;
- preparation of a false seedbed (stale seedbed) to allow spilled *Brassica* sp., sugar beet or chicory seed to germinate, followed by mechanical or chemical inactivation (systemic non-selective herbicide) before the 5-leaf stage.

In 2019 COGEM proposed methods of inactivation of *Brassica napus* plants in a field that contained the non-authorised glyphosate tolerant GT73 event (COGEM, 2019). The first option was spraying with systemic herbicides before flowering (containing no glyphosate). However, also Roundup (which contains glyphosate) could be used as the GT73 event is present in a very low amount (Table 1). The majority of the plants will be inactivated and the surviving GT73 plants can then be pulled out and destroyed. To eliminate non-germinated seed, the field needed to be harrowed (false seedbed) afterwards to allow germination. Monitoring in the following years was not advised, because of the low-level presence and because GT73 is authorised for food, feed and processing, meaning that the environmental risk of an accidental spill is negligible. Moreover, monitoring was deemed to have little value for this case in the event oilseed rape was included in earlier crop rotations.

In the advice on non-authorised petunias COGEM opted for incineration or industrial composting following the procedures for certified compost[16] to ensure also seeds are inactivated (COGEM, 2017). APHIS-USDA advised for the same type of petunia plants to use one of the following methods: incineration, municipal landfill, 30 cm deep burial, autoclaving, and, if no seeds are present, composting

The International Seed Federation (ISF) lists the following options for seeds treated with pesticides (ISF, 2014):

- sanitary landfill,
- power plant,
- cement kiln,
- waste management facility, or
- ethanol plant.

Used seed bags have to be incinerated.

Methods used for the destruction of quarantine organisms and material infested with them may be suitable as well. This matter is coordinated by the Netherlands Food and Consumer Product Safety Authority (*Nederlandse Voedsel- en Warenautoriteit, NVWA*). The methods include incineration, fermentation, composting, steaming and landfill, but only in facilities recognised by NVWA[17]. The requirements for recognition assure that also seeds will be destroyed (see Chapter 5).

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[13] "Regeling genetisch gemodificeerde organismen milieubeheer 2013, update 2019, https://wetten.overheid.nl/BWBR0035072/2019-07-01

[14] https://www.biosafety.be/sites/default/files/Protocol-voor-de-aanleg-de-opvolging-en-de-oogst.pdf
https://www.biosafety.be/sites/default/files/protosb2002n.pdf
https://www.biosafety.be/sites/default/files/Protocol-voor-de-aanleg-de-opvolging-en-het-rooien%20%2816%29.pdf

[15] https://www.biosafety.be/ or https://www.bioveiligheid.be/ or https://www.biosecurite.be/

[16] http://keurcompost.nl/wp-content/uploads/images/Factsheet-akkerbouwers-1.pdf

[17] https://www.nvwa.nl/documenten/plant/plantziekte-en-plaag/plantziekte-en-plaag-overig/publicaties/register-erkende-vernietigingslocaties-q-organismen

Although waste derived from agricultural activities is excluded from the waste management legislation[18], some recommendations may be useful. The proposed hierarchy of treating waste, i.e. preparing for re-use, recycling, recovery, and disposal, may be applied in the choice of inactivation methods. In contrast, seeds that are coated with hazardous chemicals are covered by the waste management legislation.

4.2 Preventing dispersal and volunteers

At flowering, depending on the crop, pollen may flow to neighbouring sexually compatible crops and wild type species. If cross-pollination cannot be excluded at the time the commingling is noticed, the neighbouring plants within isolation distance should be inactivated the same way as the affected crop. It goes without saying that whenever possible plants need to be destroyed before flowering.

At harvest seeds may be spilled on the soil. Also, at the initial sowing not all seeds may have germinated. Shallow cultivation to prepare a false seedbed (see 5.3.1) will allow the spilled seeds to germinate before cultivation activities for the next crop starts. The young seedlings can be inactivated with a suitable method described below. This measure is especially important to avoid seeds to become buried and dormant (secondary dormancy) for species such as *Brassica* sp.*, Beta vulgaris* and *Cichorium intybus*. Consequently, ploughing has to be avoided for species that may develop secondary dormancy.

Ploughing of a crop may be a valuable method to inactivate some plant species, but may result in volunteers in the next season for others, as is the case for e.g. potato. Another inactivation approach may be needed pre-ploughing.

Attention should be paid in case seed need to be transported for inactivation in order to avoid dispersal.

A monitoring protocol should be considered taking into account the plant species, the inactivation method and whether the commingling event is authorised for food, feed and processing in the EU, to cope with volunteers should they arise.

4.3 Transportation

Transport needs to be limited as much as possible to avoid spills along the transport routes. The plant material is preferably inactivated as close as possible to the source. Whenever transport of an unauthorised GMO is involved, the *Regeling GGO*, Annex 1, refers to the dangerous goods legislation[19]. Annex 1.2 mentions packaging instructions. Transport on public roads has to follow the ADR legislation[20]. Annex 1.2 of the *Regeling GGO* only requires “closed, break-resistant units”, from which no material can escape, whereas the ADR requires for GMOs a triple packaging (packaging instruction P904). For transport within the Netherlands, an exemption may be applied for (VLG, Art. 2[18]).

In case the seed or planting material is commingled with an event that is authorised for food/feed, transport does not need to comply with these regulations.

Finally, it is likely that some inactivation methods may not be adequately validated and/or readily available at the moment of detecting the issue. This may require a temporary storage of the material to be inactivated. Also this storage must be secured in a way that further dispersal is avoided.

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[18] EU Waste Framework Directive 2008/98/EC (https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32008L0098), Wet Milieubeheer (https://wetten.overheid.nl/BWBR0003245/2019-11-14)

[19] Wet vervoer gevaarlijke stoffen (https://wetten.overheid.nl/BWBR0007606/2015-04-01)

[20] ADR (https://www.rijksoverheid.nl/documenten/publicaties/2015/05/21/adr) Annex to “Regeling vervoer over land van gevaarlijke stoffen (VLG)” (https://wetten.overheid.nl/BWBR0010054/2019-07-01)

4.4 Monitoring

In contrast to methods that can be used for inactivation, monitoring in itself will not eliminate any material. Rather it can contribute to confirm the efficacy of the inactivation or provide a tracking system of remaining problems. After the inactivation or removal of the crop, fields may need to be monitored for volunteers to be inactivated, either originating from germinating seeds or regrowth. Not only spilled seed, but also seeds from the initial sowing that may have failed to germinate the first time, may end up in more favourable germination conditions in the subsequent crop(s).

The choice of the subsequent crop will determine the ease with which volunteers can be observed and tackled. Chemical inactivation of dicot species usually is fairly feasible in a monocot succeeding crop. In a dicot crop inter- and intra-row mechanical weeding may be a solution. Another example is a grass crop after a dicot crop followed by extensive mowing that will exhaust and devitalise the dicot volunteer.

The level of commingling will also determine whether, after a risk assessment, monitoring is advisable. Mixing rates tend to be very low (Grantina-Ievina et al., 2019)(see also Table 1), probably not justifying monitoring efforts, depending on the species and whether the commingling GM event has been authorised for food, feed and processing (COGEM, 2019) (see also 5.5).

4.5 Post-treatment clearance

During the performance of this study, the question was raised on responsibility for deciding that the treatment has been successful. Although in principal not related to the technical aspects, the authors like to flag that clarity on this aspect is important. When GMO presence is detected that leads to an obligation to remove the material, not only suitable methods should be identified, it should also be indicated who the responsible parties are (including inspection), what type of verifications will occur and what type of reporting is expected.

In comparison, for field trials with genetically modified plants, conditions for choice of subsequent crops as well as monitoring and treatment of volunteers have been imposed. In some cases, these conditions were only relieved when, in a number of subsequent seasons, no GM volunteers were observed. This information had to be included in a report to the authorities in support of the conclusion of the post-trial monitoring period.

While COGEM has advised on specific cases of handling inadvertent GMO presence in the past, the authors could not find any trace of the successful conclusion of the further handling. Nevertheless, such documented follow-up would be useful to track compliance and to learn on best practices.

5 Inactivation methods

In the following sections inactivation methods are grouped according to the primary mode of action:

- physical treatments relying on the effect of temperature, absence of oxygen and/or light;
- biological treatments requiring the action of living organisms;
- mechanical weeding based on the use of tools to pull, shred or otherwise disrupt the growing of the plants; and
- chemical treatments.

Each section describes the mode of action, the inactivation efficacy, the advantages and disadvantages. Scientific literature rarely addresses inactivation of GM plant materials specifically. Therefore, inactivation methods that are used to combat weeds in general are referred to where available. Several publications can be consulted for additional information (Ackroyd et al., 2019; Hance and Holly, 1990; Naylor, 2008; van der Schans et al., 2006).

5.1 Physical treatments

5.1.1 Autoclaving

The process uses pressurised saturated steam at 121°C for around 15 - 20 minutes depending on the size of the load and its content. Autoclaves are typically used to sterilise equipment, culture media and other laboratory supplies, as well as laboratory and greenhouse waste.

The methods can be used to inactivate seeds, plants, soil etc. For each type of load and volume the method needs to be validated, i.e. verify whether the conditions of temperature and duration are able to devitalise the plant matter.

Efficacy

If validated for the specific load, the method devitalises the plant material completely.

Pro

- This method is suitable for small volumes of plant waste; larger volumes may be processed depending availability of large equipment.
- Suitable for vegetative and generative material including soil, pots, growbags and packaging.
- The inactivated product can be discarded as household waste, industrial waste or used for composting.

Con

- Availability of a suitable autoclave (typically present in laboratories, research greenhouses and medical care centres).
- Less suitable for large volumes of plant waste as many time-consuming cycles need to be run successively to complete.
- Not allowed for seeds coated with pesticides (autoclaving hazardous materials may generate toxic vapours).

5.1.2 Incineration

Incineration is another heat treatment process used for plant waste. It involves the combustion of organic substances contained in waste materials, converting the waste into ash, flue gas and heat. Often the waste incineration plant recovers the heat energy by producing electricity.

The NVWA established a register with sites authorised to incinerate quarantine organisms[21]. As these sites comply with the requirements to inactivate quarantine organisms in a safe and contained way, they are equally suitable to inactivate plant material that contains unauthorised GM events. The very high temperatures (up to 1000°C) ensure complete inactivation. Residual ashes are transported to landfill sites.

[21] https://www.nvwa.nl/documenten/plant/plantziekte-en-plaag/plantziekte-en-plaag-overig/publicaties/register-erkende-vernietigingslocaties-q-organismen

Efficacy
The method ensures complete inactivation.

Pro
- Waste incineration plants can accept large volumes.
- Suitable for vegetative and generative material including soil, pots, growbags and packaging.
- Suitable for seeds coated with pesticides.
- Incineration produces energy (applying the waste hierarchy).

Con
- Unclear whether incineration plants would accept large volumes of material with a high water content (causes temperature drop).
- Transport over large distances may be necessary.
- Legislation on transport of dangerous goods is applicable.

5.1.3 Freezing

Freezing affects plant cells in several ways: damage of the plasma membranes due to ice crystal formation, dehydration, irreversible inhibition of photosynthesis and protein denaturation (Thomashow, 1999). The temperature threshold below which irreversible damage occurs depends on the plant species and organ (e.g. leaves vs seed) and whether the plants were allowed to acclimatise.

Freezing can be an inactivation method for many cold-sensitive plants, tissues and organs. Seeds are less vulnerable to freezing damage. Tubers (e.g. potatoes) and roots (e.g. chicory) are sensitive to freezing temperatures. Freezing was accepted for potato in COGEM advice CGM/011029-01 (COGEM, 2001). A temperature of -1.5°C in the whole load would be sufficient (pers. comm. Dr. P. Bruinenberg).

Efficacy
Freezing is an effective method to inactivate plants and plant parts that are sensitive to cold (in general not for seeds). Temperatures of -18°C as in household freezers will be sufficient. Slow freezing will favour the formation of disrupting ice crystals.

Pro
- Suitable for vegetative and generative material that is sensitive to freezing.
- Equipment might be readily available for small volumes.

Con
- Large volumes need professional freezing facilities.
- Transport over large distances may be necessary.
- Legislation on transport of dangerous goods is applicable.

5.1.4 Steaming

5.1.4.1 Seeds

Steaming seeds at atmospheric pressure (100°C and ±100% RH) is another method to devitalise seeds. This can be performed in ovens or in specially designed steam carts/containers for a period of 4h. Lower temperatures are often not sufficient (Dowsett and James, 2017). However, treating weed seeds at 85°C and 40% RH for 15h also completely prevents germination. The weed species in this investigation were *Digitaria violascens*, *Eleusine indica*, *Lepidium virginicum*, *Plantago lanceolata*, *Portulaca oleracea* and *Sonchus oleraceus*. *P. oleracea* was the toughest species, probably due to its hard seed coat.

Mobile units are available for rent[22], in this way avoiding transport of the seed lots.
Wageningen University & Research has a large steaming installation (10 m^3) that allows for large volumes to be treated.

[22] https://edepot.wur.nl/355116 and https://www.nvwa.nl/documenten/plant/plantziekte-en-plaag/plantziekte-en-plaag-overig/publicaties/register-erkende-vernietigingslocaties-q-organismen

For each type of load and volume the method needs to be validated, i.e. verify whether the conditions of temperature and duration are able to devitalise the seeds.

Efficacy
The heat inactivates all seeds, provided that a validated method is used.

Pro
- Less energy needed compared to autoclaving.

Con
- Availability of equipment, although a mobile steaming container exists.
- Otherwise, transport over large distances may be necessary.
- Legislation on transport of dangerous goods is applicable.

5.1.4.2 Vegetative material

Steam carts/containers are sometimes used in plant research and development centres to inactivate plant waste.
For each type of load and volume the method needs to be validated, *i.e.* verify whether the conditions of temperature and duration are able to devitalise the plant matter.

Mobile units are available for rent[20], in this way avoiding transport of plant material.

Efficacy
The heat inactivates all vegetative plant material, provided that a validated method is used.

Pro
- Less energy needed compared to autoclaving.
- Suitable for vegetative material including soil, pots, growbags and packaging.

Con
- Availability of equipment, although a mobile steaming container exists.
- Otherwise, transport over large distances may be necessary.
- Legislation on transport of dangerous goods is applicable.

5.1.4.3 Soil

Steaming of greenhouse soil has been used for decades to sanitise the soil (Bollen et al., 1981; Ludeking et al., 2013). Weed seeds as well as soil pests and pathogens are inactivated. A steam generator provides steam (100-130°C) via tubing underneath a sheet that covers the soil. The soil needs to be cultivated beforehand to allow a good (deeper) penetration of the steam. Usually a retention period of 6 hours is applied. A temperature between 70-80°C is aimed for at a dept of approximately 35 cm. After steaming the soil needs to rest and be allowed to build up soil life. Drain steaming makes use of drains that are permanently present at a dept of 55-60 cm. This system ensures a better penetration. Steaming under negative pressure makes use of the same type of incorporated tubing as in drain steaming but are now sucking the steam into the soil by means of a ventilator. A fourth method is steam injection with a steam injector mounted on a cultivator. The type, structure and moisture content of the soil and the groundwater level determine the efficacy.

The effect on weed seedling emergence depends on soil type, soil moisture, soil structure and heat duration (Melander and Kristensen, 2011). The effect on spring oil seed rape seeds (*Brassica napus*), perennial rye-grass (*Lolium perenne*), *Capsella bursa-pastoris* and naturally present weeds was tested in laboratory studies (9.5 L soil samples, 80 mm high). A steam treatment aiming at 60-80°C on finely structured sandy soil gives the best results. In heavy soils or soils with large clumps the steam penetration is limited. In moist conditions the cooling down period lasted longer than in dry conditions, leaving the seeds for a longer period at high temperatures. However, reaching the maximum temperature is of greater importance than the rate of cooling. Another laboratory study confirms the finding that lower temperatures are sufficient, limiting the negative effects of higher temperature, e.g. energy needed, release of manganese and ammonium from the soil and killing also beneficial microflora (van Loenen et al., 2003). Seeds of annual *Chenopodium album* were killed at 60-65°C and rhizomes of perennial *Agropyron repens* were killed within 11 minutes at 60°C. Bollen and colleagues (1981) report that <1% of weed seeds survives 57.5°C and 60°C is sufficient to kill all weed seeds. Non-imbibed seeds, as opposed to imbibed seed naturally present in moist soil, require higher temperatures to have the same effect (Melander and Jørgensen, 2005; Melander and

Kristensen, 2011). This has to be taken into account for seeds in the upper soil layer where long dry periods precede steaming, particularly on sandy soils.

As the high temperatures kill both pathogenic and non-pathogenic microorganisms, the soil need to be recolonised for a beneficial biological balance. This can take up to one year depending on the organism (Bollen et al., 1981; Ludeking et al., 2013). Minerals become available due to the high temperature. Especially manganese may induce phytotoxicity.

Soil steaming is common practice in greenhouses (soil-bound crops) and outdoors on raised beds for vegetables, primarily to control soil-borne pathogens. Farmers may apply the technique themselves or specialised companies are hired. In open fields, soil may be treated the same way, although probably only feasible on a limited area. However, mobile steaming devices are being trialled (Nishimura et al., 2015).

Growbags used in protected cultivations may be heat treated as well.
Renewi / Van Vliet BV[23], Wateringen, South Holland (multiple processing sites) can be addressed for recycling of growbags.

Efficacy
The classical method of soil steaming at 70-80°C for 6 hours is effective to inactivate weed seeds. Comparing with experimental set-ups, this temperature will allow for 99% inactivation of imbibed seeds (Melander and Kristensen, 2011). For a sown but not yet emerged crop to destroy, a depth of 100 mm will be sufficient for the effective temperature to reach.

Pro
- Suitable for small (indoor) areas.
- Performed on-farm.

Con
- Needs time and effort to rebuild soil life; requires to respect a waiting period to plant the next crop.
- Less suitable for large areas of arable crops.

5.1.5 Soil solarisation

Soil solarisation is primarily employed as an alterative for soil steaming and the application of methylbromide (no longer allowed) to disinfest soils. However, also weeds can be coped with. The covering of the soil with a layer of clear (or black) plastic, kills weeds and weed seeds due to the elevated temperatures of the soil induced by the sun (Stapleton et al., 2000). The method is most effective when implemented in the summer with moist soil conditions. It takes 3-6 weeks for a complete devitalisation. Because of the soil moist that has to turn into steam, heavy soils give best results. It may be less effective on sandy soils, which drain faster and produce less steam. However, irrigation may help. The method was developed in Israel and is practised in the Mediterranean region and the southern regions of the USA (Stapleton et al., 2000).

For experiments in Syria clear polyethylene sheets were used on an irrigated chromic luvisol[24] during July and August for different periods (Linke, 1994). Soil temperature during solarisation was measured and weeds were collected in the subsequent winter crops (lentils and faba beans). The maximum temperature recorded at 5 cm soil depth was 57°C. In plots solarised for 50 days, nine weed species (out of a total of 57 identified species) were controlled to 100%. The total number of summer weeds in the control was 24.4 plants/m^2, whereas in the 50-day solarisation this was decreased to 5.4 plants/m^2. Annuals were best controlled. Biennial or perennial species have their root and storage organs deeper in the soil and therefore are not heated to the same extent as the top layer.

The method may be applied in greenhouses for soil-bound cultivations as well as for outdoor cultivations. In the Netherlands, the method may be considered only in greenhouses, but the long

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[23] https://www.renewi.com/nl

[24] High-activity clays, high base status, yellow-red (World Reference Base for Soil Resources, https://www.boku.wzw.tum.de/index.php?id=wrb

incubation period limits the applicability (Ludeking et al., 2013). A combination of solarisation and anaerobic soil disinfestation (see 5.1.6.1) may improve results.

Efficacy
Most effective under climatic and weather conditions of high air temperature and long days for soil heating. Annuals in the top soil layer are most sensitive.

Pro
- Uses the sun's energy and avoids extra (fossil) energy consumption once placed.
- Performed on-farm.
- Suitable on (vegetable) farms where machinery to apply the transparent film is available.

Con
- Only possible in hot weather, less suitable in the cooler seasons. Often not feasible at normal sowing times for most arable crops.
- Requires disposal of used plastic film.
- Long treatment period
- Risks of phytotoxicity, when the next crop is sown or planted
- Less suitable for sandy soils.

5.1.6 Biological soil disinfestation

Biological soil disinfestation is a method based on anaeroby to control soil-borne pathogens, plant-parasitic nematodes and weeds (Blok et al., 2000; Gioia et al., 2016; Lamers et al., 2010; Meijer and Lamers, 2004; Shrestha et al., 2016). The method comprises the incorporation of organic matter in the soil, irrigation to soil saturation and then covering with plastic film. Another method to create an oxygen-free soil layer is inundation.

5.1.6.1 Anaerobic soil disinfestation

The organic matter should be readily decomposable (grass, potato haulms, crop residues, wheat bran, green manure, …) and finely dispersed in the topsoil. The soil is pressed, irrigated and covered with oxygen impermeable plastic. Decomposing microorganisms deplete the topsoil of oxygen within a few days, followed by anaerobic decomposition by facultative anaerobes. The plastic film and soil saturation maintain the anaerobic conditions and stimulate the anaerobic decomposition. In this process toxic/suppressive substances are produced (e.g. O_2, NH_3, H_2S, CH_4, and N_2O, volatile organic compounds, such as butyric acid and acetic acid) (Runia et al., 2014; Shrestha et al., 2016). The method is particularly interesting when locally-sourced waste products can be used as carbon source. About 40 tonnes of fresh material is needed per hectare (Lamers et al., 2010).

Most of the studies report on the effects on pathogens and nematodes, few on weeds. In a meta-analysis of different types of anaerobic soil disinfestation studies on the impact on weeds, the overall weed reduction was 63% (weed count and germination percentage) (Shrestha et al., 2016). A high soil temperature (>35°C) and long incubation periods (>10 weeks) were more suppressive (experiments conducted in pots) (Muramoto et al., 2008). Weeds at shallow depts (0-5 cm) are more affected than at moderate depts (6-15 cm)(Shrestha et al., 2016). The shallow dept as defined in this study nicely corresponds to the normal seeding dept of most crops. In greenhouses higher temperatures can be achieved compared to outdoors.
Roots, rhizomes, bulbs and plants show better results than seeds, they seem to be less resistant to anaerobic conditions than seed (Meijer and Lamers, 2004).

In outdoor experiments in 2 sites in Florida in spring, anaerobic soil disinfestation with a mix of composted poultry litter and molasses for 3 weeks were compared with chemical soil fumigation (Di Gioia et al., 2016). In one site the efficacy on weed inactivation was 100% (weed coverage) for the chemical and on average 85% for the poultry litter/molasse treatment. The other site had a higher initial weed pressure and none of the treatments provided acceptable weed control.

Commercial soil amendments are available (Herbie from Thatchtec BV[25]) that speed up the process of inactivation of nematodes (from 6 weeks to 2-3 weeks) with similar but more reproducible results (Ludeking et al., 2013; Runia et al., 2012; Runia et al., 2014). Also, seeds (*Stellaria media*) were

[25] www.thatchtec.com

successfully inactivated using Herbie 7025 in greenhouse conditions (100% inactivation after 15 days) (Ludeking et al., 2013).

Efficacy
Suppresses soil-borne pathogens, plant-parasitic nematodes and weed seeds. Results vary with the type of amendment, environmental conditions and species.

Pro
- Suppresses weed seeds in an environmentally friendly way (as compared to chemical or steam disinfestation methods).
- Performed on-farm.
- Best results in greenhouse conditions.
- Adds to soil fertility due to the incorporation and decomposition of organic matter (Di Gioia et al., 2020).

Con
- Best practised in summer; often not feasible at normal sowing times for most arable crops.
- Long treatment period.
- Risks of phytotoxicity, when the next crop is sown or planted (Di Gioia et al., 2020).
- Varying results depending on temperature, type of amendment and soil.
- Requires disposal of used plastic film.

5.1.6.2 Inundation

Flooding an area to be water-saturated at a depth of 15 to 30 cm for a period of 3 to 8 weeks is another method to control weeds (de Kool, 2008; Monaco et al., 2002). Plant roots are killed due to a lack of oxygen. But flooding may have little effect on seeds in the soil. Different species vary considerably in the level of oxygen required for seeds to germinate. Also, secondary dormancy may be induced, e.g. in *Brassica napus* (Pekrun et al., 1997).
Results are best at soil temperatures >17°C and operations are therefore preferably performed in summer (de Kool, 2008).

Cirsium arvense, *Tussilago farfara* and *Elytrigia repens* are well managed by inundation, and *Rorippa sylvestris* to a lesser extent (de Kool, 2008). Inundation is not effective to suppress *Equisetum arvense* and *Cyperus esculentus*.

Efficacy
Next to annual plants, very effective against perennial species.

Pro
- Kills annual as well as perennial plants.
- Performed on-farm.

Con
- Low effectiveness on seeds.
- Long treatment period.
- Restricted to soils with a low water infiltration rate underneath (Lamers et al., 2010).
- Requires creating dikes and field levelling.
- Disturbs the biological balance in the soil, reducing the choice of next crops (de Kool, 2008).
- May induce nutrient leakage.
- Availability of water; water from rivers may introduce weed infestations next to other polluting substances.

5.1.7 Mulching

Mulch impedes the emergence of weeds, and may be applied when an already sown, but not emerged crop needs to be destroyed. It affects seeds by excluding light and providing a physical barrier for emergence.

Several covering options are available: biodegradable films, straw and compost (van der Schans et al., 2006). Usually this technique is practised between crop rows. Organic material gradually decomposes, but stays effective at least until crop closure. Teasdale and Mohler (2000) compared 7 types of mulches and several thicknesses in a study of the relationship between physical properties and the germination of *Abutilon theophrasti*, *Setaria faberi*, *Chenopodium album*, and *Amaranthus*

retroflexus. The mulches were *Zea mays* stalks, *Secale cereale*, *Thifolium incarnatum* and *Vicia villosa* residues, *Quercus* leaves, bark chips, and landscape fabric strips. Weed emergence was observed for 10-12 weeks. Most seedlings were observed within a month after set-up of the experiments. As expected, the thicker and denser the mulch the better the weed suppression. However, for *A. retroflexus* emergence was stimulated at low mulch rates, associated with an increase of nitrate that stimulates dark germination in this species. Weeds with small seed sizes were more sensitive to emergence suppression, probably because of lower seed reserves to grow in the dark.
In case this inactivation method would be chosen for an already sown, but not emerged crop, the choice for a next crop is potentially limited to one that is planted instead of sowing, because of the long treatment period. Otherwise, a complete growing season is missed.

Efficacy
Efficacy depends on the type of material and thickness of the mulch layer (Teasdale and Mohler, 2000). Both elements determine the light transmittance and physical barrier. Germination can be suppressed provided that the mulch is present for a sufficiently long period.

Pro
- Natural materials may add nutrients to the soil.
- Performed on-farm.

Con
- May be a costly option.
- Long treatment period.
- May limit possibilities for an alternative next crop to be sown or planted.
- The mineral supply may cause manure legislation issues.

5.1.8 Heat weeding

Weeding may be applied, once plants have emerged. In this section the use of heat is described, in section 5.3, mechanical methods are explained.

5.1.8.1 Flame weeding

Thermal weed control may be applied using a flame weeder. This technique relies on intense temperatures to rupture plant cells and rapidly kill plant tissue (van der Schans et al., 2006). Using open flame tools is most effective. Dicot seedlings are preferentially treated in the cotyledon to 4-leaf stage, monocot seedlings in the cotyledon to 2-leaf stage. The method will not impact underground vegetative structures or weed seeds in the soil, as heat is not transferred into the soil. Dry leaf surfaces are more likely to be damaged than moist or wet leaves (Ackroyd et al., 2019).

Field experiments with *Sinapis alba* showed that to control 95% of the plant number 1.75 as much energy was needed in the 2-4 leaves stage compared to the 0-2 true leaves stage (Ascard, 1994).

5.1.8.2 Hot water weeding

Hot water is also used to treat weeds and could be an alternative. The technique is mostly applied on hard surface areas and on railway embankments (Hansson and Mattsson, 2003). The purpose is to heat the growing tip to >65°C resulting in the plant dying-off. Best suited for small weeds, larger plants require multiple applications to exhaust the plants ability to recover (Hansson and Ascard, 2002). To kill 90% of *Sinapis alba* test plants requires 2.7x more energy at the 6-leaf stage compared with the 2-leaf stage. For a 90% fresh weight reduction the factor is 2.9.

Hot water weed control shows best results carried out when the plants are drought stressed (Hansson and Mattsson, 2003). Results are not as good when the plants are wet. In these experiments again *Sinapis alba* was the test species to calculate the energy/m^2 needed to reach a 90% reduction in fresh weight. The air temperature has a minor influence on the weed control effect (7°C and 18°C were tested).

Empas[26], Veenendaal, province of Utrecht, delivers thermal weed control machinery. Machinery for both local or full-field application exists. (Heatweed Technologies AB[27] located in Ede, Gelderland, delivers technology especially for surface applications, that can be used in agriculture as well).

Research is still on-going to develop machinery especially for organic farmers (Spagnolo et al., 2019).

5.1.8.3 Steam weeding

Another method that has been investigated uses a mobile surface steam applicator (Kolberg and Wiles, 2002). In this publication the steam applicator measured 1.9 m long by 2.4 m wide and comprised six rows of eight nozzles (30 cm spacing) per row. The water temperature just prior to application averaged 175°C. Two speeds, and therefore application rates were compared. The effects were best for young seedlings and at a slow speed (3,200 kg/ha steam at 0.8 m/s). Measured 4 weeks after application the efficacy for *Chenopodium album* seedlings was almost 100% and at the 4 to 6-leaf stage about 95%, and for *Amaranthus retroflexus* this was about 95% and 63% resp. In a mixed weed stand the dry weight measured 9 weeks after application, the high dose was only slightly better than the low dose: ±27% reduction and ±16% compared to control. Steam application in combination with tillage did not inhibit the emergence of weed seeds with this set-up.

Efficacy heat weeding

Works best on small broad-leaved plants. Less effective for grasses, because the growing point is located at or below the soil surface.
The required treatment interval with hot water and flame weeding is, on average, 25 days on well-established natural weeds on a gravel embankment (Hansson and Ascard, 2002)

Pro

- Does not disturb the soil.
- Performed on-farm.

Con

- Dependent on fossil fuel to generate flames, hot water or steam on top of pulling machinery.
- Not suitable for soil covered seeds.
- Less effective for grasses and perennial species.

5.1.9 Deep burial

Deep burial is allowed for plant material infected with quarantine pests, but only at official pits, authorised for the purpose. The burial then consists of dumping and immediately covering with at least 1 m of other material. Deep burial is practised in GM field trial management to inactivate leftover seed (Czech Republic, Germany, Romania, Spain) or other GM material. At least a depth of 50 cm is required to devitalise seeds. Deep burial means at least below the depth that is usually reached by ploughing and other cultivation machinery.

Efficacy

Effective to destroy seeds, provided the pit is deep enough.

Pro

- May be performed on-farm.
- Small seed lots may be inactivated by digging a pit; large seed lots by deep ploughing.

Con

- Large quantities of material require speciality machinery (contractor).
- Deep ploughing may disturb the biological balance and physical characteristics of the field.

5.1.10 Burning on the field

A crop that already has set seed, instead of being harvested and the seed inactivated, alternatively may be burnt on the spot[28]. Seeds that are already dispersed may escape, but seeds on the plant

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[26] https://www.empas.nl/en/solutions/thermal-weed-control

[27] https://heatweed.com/nl/methode-nl/

[28] Seeds from some tree species need a fire to germinate. The hard seed coat is water impermeable. Seed coat destruction by fire allows the water to penetrate and start the germination process. So far, no such species are relevant for the Netherlands.

often are completely destroyed (Monaco et al., 2002). Results are best when the harvested material is swathed (narrow windrow burning), in order to have dense burning material to increase the heat and exposure time (Ackroyd et al., 2019).

Burning on the field is also recommended as an inactivation method in GM field trials. However, burning in general is prohibited by the Dutch environmental legislation (*Wet milieubeheer*[29]) Art 10.2. An exemption may be granted by the local mayor (Art. 10.63). On the other hand, the *Uitvoeringsregeling rechtstreekse betalingen Gemeenschappelijk LandbouwBeleid*[30] stipulates that when a farmer applies for a direct payment, he has to comply with the standards for good agricultural and environmental condition of land. The standards are listed in Annex 4 of the *Uitvoeringsregeling* and include the ban on burning arable stubble, except for plant health reasons. As a consequence, burning on the field of plant material mixed with unauthorised GM material is not possible.

Efficacy
Effective to destroy seeds on the plant when high temperatures (e.g. 400°C) can be guaranteed for a sufficient period (e.g. 30 seconds).

Pro
- Performed on-farm.
- Avoids shattering of mature seeds, if applied at the right moment.

Con
- Probably not legal as inactivation method.
- Small window of applicability: not feasible under high humidity conditions, high winds and rainfall.
- Seeds that already lie on the surface, may remain unaffected, depending on the intensity of the heat.
- Not suitable for perennial plants as the underground part is not inactivated.

5.2 Biological treatments

5.2.1 Composting

Composting turns organic material into a humus-like material, i.e. compost. The decomposition process is performed by composting organisms such as earthworms and other detritivores, fungi and aerobic bacteria. The method requires oxygen (aerobic method), moisture, a correct pH and C/N ratio. Shredding the plant matter beforehand may aid. To maintain optimal processing conditions the organic mixture is regularly turned. This may be achieved by mechanical turning and mixing introducing air in controlled bioreactors.

The process starts with mesophilic organisms that operate at temperatures of 20-45°C (Singh et al., 2006). The first stages of composting are acidic, after that pH rises, but so does temperature killing most mesophilic organisms. After that thermophilic microorganisms take over. They function between 45 and 70°C. Above 55°C for 3-15 days most pathogens and weed seeds are killed.

An industrial-scale, controlled system, which ensures these conditions are uniformly achieved, is suitable for GM plant material inactivation. Again, the NVWA has a list of composting facilities[21] that are approved to inactivate quarantine organisms and therefore also suitable for inactivation of GM plants and seeds.

Keurcompost-certified composting plants have to comply with stringent product requirements[31]. These requirements may be used as guidelines for open field composting (mainly used for plant material) or indoor composting (mainly used for vegetable, fruit and garden waste). For every type of material that needs to be inactivated, the suitability of the different systems needs to be checked. A list of certified plants is available at the Keurcompost website[32].

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[29] https://wetten.overheid.nl/BWBR0003245/2019-11-14
[30] https://wetten.overheid.nl/BWBR0035925/2020-02-26
[31] http://keurcompost.nl/beoordelingsrichtlijn/
[32] http://keurcompost.nl/locaties/

Compost can be used as an additive to soil providing humus and nutrients. It is an absorbent material that holds moisture and soluble minerals.

Efficacy
If a high operating temperature can be guaranteed lasting for a sufficient period, composting is a suitable method for inactivating seeds. The requirements for inactivation of vegetative material only are not as demanding.

Pro
- Composting plants can accept large volumes.
- Suitable for vegetative and generative plant material, provided the method for Keurcompost is guaranteed.
- Applying the waste hierarchy, producing compost.

Con
- Transport over large distances may be necessary.
- Legislation on transport of dangerous goods in applicable.
- Not suitable for material containing soil, pots or packaging; sorting out non-organic material may be necessary.
- Composting emits methane (greenhouse gas) (if not used as fuel).

5.2.2 Fermentation - Anaerobic digestion

Anaerobic digestion is a process where microorganisms break down organic material in the absence of oxygen. First the material is hydrolysed to split complex organic molecules into simple sugars, amino acids, and fatty acids, that digesting bacteria can access. Further stages finally result in the production of carbon dioxide (CO_2) and methane (CH_4). The output of fermentation plants is bioenergy via methane (biogas). The nutrient-rich digestate can be used as fertiliser.

Concerning temperatures two systems are used:
- Mesophilic digestion, where mesophiles are the primary microorganisms operating optimally around 30 to 38°C;
- Thermophilic digestion, where thermophiles are the primary microorganisms with an optimum around 49 to 57°C.

With the mesophilic system weed seeds may only be killed after several days. Germination experiments were conducted on weed seeds treated at 37°C mixed with cattle manure (Johansen et al., 2013). *Avena fatua*, *Sinapis arvensis*, and *Solidago canadensis* failed to germinate after 2 days of batch digestion. *Brassica napus, Fallopia convolvulus* and *Amzinckia micrantha* still maintained low levels (±1%) of germination ability after 1 week. For *Chenopodium album* 7% still germinates after 7 days. At 55°C none of these test species germinated after 2 days. Another experiment simulated fermenter conditions of 42°C, at pH 7 in a water bath (up to 18 days) and an experimental digester (up to 36 days) (Hahn et al., 2018). For 6 of 11 wildflower species tested inactivation was very slow (mean inactivation time >7 days in the water bath) or even lacking. *Cynodon dactylon* and *Melilotus officinalis* survived. The results show that in these conditions for 6 species dispersion via the digestate is possible.

It is not clear which type (mesophilic or thermophilic) fermentation plants are listed on the NVWA list[21]. Residence time is equally important.

Again, the Keurcompost requirements for fermentation[31] may be used as a guideline, but the suitability of the different systems needs to be checked for every type of material.

Some more data are mentioned by Elema and Scheepers (1992). Anaerobic fermentation of cattle manure at 35°C can inactivate seeds already after 4 days (*Avena fatua*, *Brassica napus*). For *Chenopodium album* 5 weeks are needed. In pig manure seeds devitalise quicker than in cattle manure. Seeds in a fermenting vegetative plant mass can survive 6 weeks (*Echinochloa crus-galli*).

When the digestate is stored afterwards, the germination capacity of any surviving seeds is further reduced, as is also the case for otherwise untreated manure (see 5.5).

Farmers may have installed a micro-scale biogas installation at their farm processing waste streams of the farm, such as manure and crop residues (Hjort-Gregersen, 2015). These installations are usually linked to livestock farms and are predominantly used to process manure. Several types of installations are available. The process temperature is usually mesophilic or may be thermophilic. Retention time varies between 25 and 40 days. In case also material other than manure is used, the hydraulic retention time of 150 days may be advised, with an additional digestate storage period of 180 days. In most cases biogas is converted into electricity and heat.

Micro-scale biogas installations may be useful for the inactivation of a crop in the vegetative phase on the same or neighbouring farm (to reduce transport). It would be less suitable for seed-bearing plants, as seed inactivation is not always guaranteed.

Efficacy
If a high operating temperature (thermophilic process) can be guaranteed lasting for a sufficient period of time, anaerobic digesting is a suitable method for inactivating seeds. The requirements for inactivation of vegetative material only are not as demanding.

Pro
- Fermentation plants can accept large volumes.
- Suitable for vegetative and generative plant material, provided thermophilic digesting.
- Possibility to ferment on-farm using small digesters, without major transport costs.
- Applying the waste hierarchy, producing biogas.

Con
- Transport over large distances to industrial fermentation plants may be necessary.
- Legislation on transport of dangerous goods is applicable.
- Not suitable for pesticide treated seeds.

5.2.3 Fermentation – Ethanol production

The first generation of bioethanol producers use grains, sugar beets and potatoes. Cellulosic bioethanol production plants (second generation) use non-edible raw materials like wood, switch grass, bagasse, forestry and agricultural waste. Complex carbohydrates, such as lignin, hemicellulose and cellulose are first treated using enzymes, steam heating, or other pre-treatments to free the sugars that then can be fermented to produce ethanol. The by-product of grain ethanol is protein-rich dried distillers' grains with solubles (DDGS) to be included in animal feed, next to electricity and CO_2. The steam heating step in the process ensures that seeds are inactivated.

The DDGS can only be used in animal feed provided that the GM event was identified as an event that is authorised in the EU for feed use. Likewise, ethanol plants cannot use pesticide-treated seeds in the fermentation process if the distillers' grain or mash is sold as animal feed, or when used in agriculture as soil amendment.

The largest industrial bioethanol producer is located in Europoort Rotterdam (Alco Energy Rotterdam, South Holland), mainly using maize. An example of a bioethanol production installation on-farm can be found at Maatschap Bosma in Zuidvelde, Drenthe (EOS-Demonstration projects[33]). Biofuel initiatives can be found in the "*Catalogus van Nederlandse biobrandstofinitiatieven*"[34].

Efficacy
The production process conditions devitalise all plant material.

Pro
- Fermentation plants can accept large volumes.
- Suitable for vegetative and generative sugar and starch containing material, as well as wood, straw, etc.
- DDGS is suitable for feed, if the GM event is approved for animal feed.
- Applying the waste hierarchy, producing bioethanol.

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33 http://adbrevio.nl/projecten-ethanolbosma/
34 https://www.sn-gave.nl/voorbeeld_all.asp

Con
- Unclear whether the fermentation plants would/can accept material outside their regular supply.
- Transport over large distances to industrial plants may be necessary.
- Legislation on transport of dangerous goods is applicable.
- DDGS not suitable for feed, if the GM event is not approved for animal feed.
- Not suitable for pesticide treated seeds.

5.2.4 Biodiesel production

Biodiesel is made by chemically reacting lipids such as animal fat (tallow), or vegetable oil with an alcohol, producing a methyl, ethyl or propyl ester. Rapeseed and soya bean oils are the most commonly used vegetable oils. A by-product of the transesterification process is the production of glycerol.

Oil is extracted from the grains in the classical process yielding oil and meal that can be used in animal feed. Crushing, high temperatures and chemicals will inactivate all grains. The meal is used as a component in animal feed.

Biofuel initiatives can be found in the "*Catalogus van Nederlandse biobrandstofinitiatieven*"[35]. Examples are:
- Biopetrol Rotterdam B.V., Rotterdam, South Holland (including Dutch BioDiesel B.V., Rotterdam, South Holland),
- Sunoil Biodiesel BV, Emmen, Drenthe (including the facilities of the former Biodiesel Kampen BV).

Efficacy
The heat and chemicals (hexane) used in the oil extraction process inactivate all seeds.

Pro
- Suitable for oil containing seeds (soya bean, oilseed rape, sunflower, …).
- Biodiesel plants can accept large volumes.
- The derived meal is suitable for feed, if the GM event is approved for animal feed.
- Applying the waste hierarchy, producing biodiesel.

Con
- Unclear whether they would/can accept outside their regular supply.
- Unclear whether they accept seeds or just oil.
- Transport over large distances to industrial plants may be necessary.
- Legislation on transport of dangerous goods is applicable.
- The derived meal is not suitable for feed, if the GM event is not approved for animal feed.
- Not suitable for pesticide treated seeds.

5.3 Mechanical treatments

5.3.1 Mechanical weeding

A wide range of machinery is available to manage weeds in arable and vegetable crops (Ackroyd et al., 2019; van der Schans et al., 2006).

A **harrow** ("*wiedeg*") uproots seedlings and covers them with a thin layer of soil. The optimal working depth is 2-3 cm. A harrow is most effective for dicot seedlings in the cotyledon to 2-leaf stage. It is not suitable for clumps of grass and perennial species or seed-bearing plants.

A **hoe** ("*schoffel*") cuts off plants 1-2 cm below the surface. The optimal working depth is 1-2 cm. The method is effective for seedlings in the cotyledon to 4-leaf stage, also effective against large well-rooted weeds and grasses.

A **rotary cultivator** ("*spitfrees*") also uproots and covers weeds with soil. It is effective from cotyledon to 15 cm.

[35] https://www.sn-gave.nl/voorbeeld_all.asp

All three applications can be used full-field and need a soil that is not too wet to yield good results. It is important that the top soil can dry to desiccate the loosened and cut seedlings (van den Brand, 1986). If the soil is compact, a combination of hoeing and harrowing may help.

Brush weeders ("*borstelwieder*" or "*onkruidborstel*"), **inter-row cultivators** ("*interrijwieder*"), **finger weeders** ("*vingerschoffel*" or "*vingerwieder*") and **torsion weeders** ("*torsieschoffel*" or "*torsiewieder*") are designed to work the soil in and between crop rows. Also hoes and rotary cultivators may be adapted to cultivate between the rows.

In case of a crop that may contain unauthorised GM plants, the whole crop can be inactivated using one of these options. The intra- and inter-row options may be suitable against volunteers in the next crop.

In case of grain crops that easily shatter, a false seedbed (stale seedbed) can be prepared using harrows and full-field hoes to allow for the dispersed seeds to germinate and to destroy them before the next crop is sown (Naylor, 2008; van der Schans et al., 2006). The cultivation depth should not exceed 2 cm, as deeper burial can prevent germination and even induce dormancy. However, this technique will reduce the number, but not eliminate all shattered seeds. In experiments to reduce the weed *Galinsoga parviflora*, present in the seedbank and germinating from the top soil, a false seedbed was prepared followed by several treatments with a rotary harrow ("*rotorkopeg*") (Riemens et al., 2011). This combination left, depending on the season, 1% to ±20% of the seeds unaffected compared to untreated plots.
The technique can be used for any spilled seed after harvest. Results may be improved by harrowing several times, alternately stimulating germination and inactivating seedlings, but only in optimal weather conditions (van den Brand, 1986). The length of the period between harvest and sowing of the next crop will determine the possibilities and therefore the inactivation efficacy.

Efficacy
If applied in good conditions, the method is fairly effective.

Pro
- Avoids the use of herbicides.
- Best on small plants.
- Performed on-farm.

Con
- Not suitable in wet conditions.
- Not suitable for rhizome forming species or with other underground storage organs, although a rotary tooth cultivator or a rotary tiller are able to lay grass rhizomes on the ground (van der Schans et al., 2006).
- Less suitable for bigger grasses (depending on type of machinery).
- May need several successive operations.
- Increases evaporation of soil moisture.

5.3.2 Mowing

Mowing cuts and destroys the above ground plant mass. Repeated mowing depletes the carbohydrate reserves in the roots and prevents seed production (Ackroyd et al., 2019). This method may be effective for species that do not sprout or regrow from stem or root segments. Mowing is more effective on annuals that start flowering than on plants still in the vegetative stage. Monocots such as grasses and cereals can withstand mowing and are therefore not to be treated in this way. The clippings can stay on the soil and desiccate, provided that no seed has been formed.

Efficacy
Best for annuals that start flowering and do not sprout or regrow from stem or root segments.

Pro
- Does not disturb the soil.
- Very effective on a limited number of plant species.
- Performed on-farm.

Con

- Not suitable for grasses and cereals.
- Requires repeated mowing and therefore takes time to complete.

5.3.3 Chopping and ploughing

Larger crops can be chopped first and then incorporated into the soil. Flail cutters or flail mowers ("*klepelmaaier*") and disk mowers ("*schijvenmaaier*") can do the job, or otherwise harvesting machines such as a combine may be used. Flail cutters and disk mowers leave the plant material on the surface. Forage harvesters and combines normally collect the harvested material, but could be adjusted to distribute the cut material over the field, if needed.

Ploughing used as pre-plant tillage chops and incorporates crop residue from the previous crop, destroys emerged weeds and buries weed seeds below a depth needed for germination and emergence (Ackroyd et al., 2019). This can be done using a mould-board plough ("*kerende ploeg*") that inverts the soil surface. A chisel plough ("*beitelploeg*") is also used for deep tillage but does not turn the soil. Disc harrows ("*schijveneg*") cut and mix. They can be used to control small weeds. Both chisel ploughs and disc harrows are not as effective to inactivate plants (Ackroyd et al., 2019).

A spading rotary cultivator ("*spitfrees*") also works plant residue into the soil. It can be combined with a flail cutter to cut and incorporate the plant material in one working passage, as often used to process green manure crops.

As far as seeds are concerned, seed burial is effective to prevent germination. Small seeds are already inhibited at rather shallow depts as they lack the energy reserves to emerge from deep within the soil. Larger seeds have to be buried deeper. Deeper in the soil temperatures are cooler, less oxygen is available and light does not penetrate. There, many seeds gradually loose germination ability and decay. However, not all seeds will lose viability and they may germinate once again in the upper soil layers after the next ploughing activity. Also, these adverse conditions may induce secondary dormancy in some species, *e.g.* oilseed rape seeds.

Efficacy

Machinery that chops plants into small pieces and buries vegetative parts and seeds are most effective. Dry soil conditions and warm weather are optimal conditions. Plants with tubers, stolons or rhizomes are less to not affected.
Efficacy is highest for plants in the vegetative stage, but also seed-bearing plants of species that have their seed easily decayed (e.g. maize). Other crops will need monitoring for volunteers in the seasons following the inactivation.

Pro

- The combination of chopping and ploughing can devitalise large plants. A mould-board plough is best suited for incorporating plant material into the soil.
- Chopping and mixing residues facilitates decomposition.
- No waiting time for the next crop to be sown or planted (but ploughing needs to be followed by more shallow tilling and rolling to prepare the seedbed and compact the soil).
- Performed on-farm.

Con

- Disturbs soil life.
- Increases evaporation of soil moisture.
- Works contrary to creeping perennial plants.
- Incorporating seed-bearing plants needs monitoring after the next soil turning operation depending on the species.
- Unsuitable for species prone to develop secondary dormancy.

5.4 Chemical treatment

Many options exist to control weeds using herbicides. It is important to know the "mode of action" of the available herbicides (Ackroyd et al., 2019; Naylor, 2008).

Pre-emergence or soil-applied herbicides control weeds from the emergence stage to emergence from the soil. They do not affect seeds as such. These herbicides have residual activity from 4 to 6 weeks depending on soil and weather conditions. As a consequence, a waiting period needs to be respected before the next crop can be sown.

Post-emergence or foliar-applied herbicides control emerged and growing weeds.

A distinction is made between contact and systemic herbicides. Contact herbicides only injure or kill the part of the plant that made contact with the chemical, whereas systemic herbicides are absorbed by the leaves or roots and are translocated to the rest of the plant's tissues. Contact herbicides are effective against annual species. Systemic herbicides also kill perennial species. For optimal effect spraying needs to be performed under optimal growing conditions ("growing weather"). Translocation under dry or cool weather conditions will be limited. To avoid injury to neighbouring crops drift, runoff, as well as leaching to groundwater (effect on aquatic organisms, human health) needs to be avoided.

To eradicate plants without discriminating, broad spectrum herbicides are used. Often GM plants are made tolerant for specific broad-spectrum herbicides. In case the GM event is known, another non-selective herbicide has to be chosen. With stacks with multiple herbicide tolerances, this might be a challenge. Selective herbicides may be used as well.

Each herbicide registration is accompanied with legal conditions of use. Herbicides are registered for a certain use, and may be applied only in certain crops. Applying herbicides to eradicate a crop that is mixed with an illegal GM event may require to apply for an exemption. Regulation (EC) No 1107/2009 on plant protection products[36], art 53, as well as the *Wet gewasbeschermingsmiddelen en biociden*[37], Art. 38 allow for the authorisation "for a period not exceeding 120 days, the placing on the market of plant protection products, for limited and controlled use, where such a measure appears necessary because of a danger which cannot be contained by any other reasonable means". A derogation can be asked at NVWA[38]. Authorised herbicides and their registered uses can be found at the CTGB's website[39].

Although registered herbicides have undergone a thorough safety evaluation concerning environmental impact, ecological and human risks, Directive 2009/128/EC[40] on sustainable use of pesticides promotes the use of alternative, non-chemical approaches or techniques.

Efficacy
The size of the plants matters: the larger the crop, the poorer the effectiveness or the higher the dose required. Larger plants can 'protect' smaller ones (umbrella effect).

Pro

- Convenient, economical and effective: one application may be sufficient, if treated at the right developmental stage.
- Does not disturb the soil.
- Allows reduced tillage and therefore minimises soil erosion.
- Performed on-farm.

Con

- Against the call for reduction of pesticide use.
- Effects on the next crop: herbicide carryover on successive plantings when persisting herbicides are used, e.g. ALS inhibitors.

[36] Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OJ L 309, 24.11.2009, p. 1.

[37] https://wetten.overheid.nl/BWBR0021670/2020-01-01

[38] https://www.nvwa.nl/onderwerpen/gewasbescherming/aanvragen-vrijstelling-voor-gewasbeschermingsmiddelen

[39] https://toelatingen.ctgb.nl/nl/authorisations

[40] Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 establishing a framework for Community action to achieve the sustainable use of pesticides. OJ L 309, 24.11.2009, p. 71.

5.5 Feed production

The scope of this report is seeds for sowing, but depending on the GM event(s) that are commingled with conventional seed, it may be possible to redirect the seed lot from seeds for sowing to grains for (human or) animal feed, if the GM event is authorised to be used as such in the EU. Although most of these grains will be inactivated during (food or) feed production and/or the passage through the gastrointestinal system, some might survive and end up in manure. This section is devoted to seeds in feed that may be dispersed into the environment via animal feed and manure.

In the risk assessment prior to market authorisation for food, feed and processing, the environmental aspects of seeds spills and dispersal via manure is already covered. Only if this risk is found to be negligible the GM event is authorised. Nevertheless, dispersal of seeds via manure - weed seeds in general, but also crop seeds - deserves attention.

Roughage (grass, silage maize, etc.) is in general the most important source of weed seeds (Elema and Scheepens, 1992), as well as crop seeds. Ensilaging reduces the viable seed load in time. Experiments show that after 12 weeks the seeds of most species were no longer able to germinate. Also grinding and pressing of feed components in the feed industry reduces the viability of seeds, although to a minor extent (Elema and Scheepens, 1992).
Grass and soft-coated broadleaf seeds are more easily destroyed in digestion than hard-coated seeds (Kaovich et al., 2005). In general, the survival of weed seeds in cattle feed decreases significantly after passage through the animal's digestive system: the fatality rate ranges between 70% and 95% (Elema and Scheepens, 1992; van der Schans et al., 2006), meaning a substantial amount of seeds are still able to germinate. Although bird feed is often not processed, chickens destroy weed seeds more effectively, because of the grinding in their gizzards, leaving a very small amount intact (Kaovich et al., 2005). To solve this remaining seed problem, Van der Schans et al. (2006) advise to:

- Store liquid manure for at least 8 weeks before applying it to the land (up to 16 weeks when stored at low temperatures).
- Compost solid manure.

Storing liquid cattle manure for 16 weeks at low temperatures (4, 12 or 18°C) hardly reduces the germination capacity for *Chenopodium album* and *Solanum nigrum* (Elema and Scheepens, 1992). In a similar experiment the oilseeds (Sinapis arvensis and *S. alba*) lost germination capacity within 2 weeks. Most *Abutilon theophrasti* seeds survived even 32 weeks. Heating for a short period (15 minutes at 75°C or 100°C) drastically reduced germination capacity.

A third method to reduce seeds in manure is the anaerobic digestion as described in 5.2.2. Chicken manure is often burnt, leaving no seed alive.

6 Assessment of the suitability of inactivation methods for each plant group

6.1 General

In Chapter 3 the characteristics of plant species that, as a GMO, may be potentially mixed with coventional material, are summarised. In Chapter 5 the characteristics of several inactivation methods are described. Taking these elements together with the plant stage (seed, vegetative, generative) at which a commingling is reported, an assessment can be performed of efficacious inactivation methods. An example is mowing: it is a suitable method to eliminate annual dicots, but not monocots, because monocotyledons have their growing point near the soil surface. The grouping of plants with the same or similar biological characteristics relevant to this report, allows to discuss inactivation options for the whole group. Options are therefore suitable to all species within the group, unless otherwise indicated.

Next, the feasability has to be taken into account. For example, autoclaving is a very effective method, but is in many cases impracticable because of the large volume of material to be processed, the availability of the equipment, transport and operating costs, etc. These considerations determine suitability.

Also, the level of commingling can play a part in this evaluation. Based on a risk assessment, very low levels may ultimately be found to have no or negligible risk for the environment. This may be the case especially when the commingling event is approved for food, feed and processing in the EU and this may justify taking no action or not performing monitoring after inactivation, especially if the species cannot persist or has no sexually compatible wild relatives in the Netherlands.

Sometimes a seed lot of one species is mixed with an unauthorised event of another species e.g. in Switzerland imported wheat was found to be commingled with a low level amount of GM oilseed rape (Schulze et al., 2015). In that case the decision tree of the GM species has to be followed.

The result of this analysis can be summarised in a decision tree listing the options. A general decision tree is presented in Figure I of theSummary. In the next sections the inactivation options for each of the plant groups as defined in Section 3.2 are discussed. Each plant group is discussed as a stand-alone section, allowing users to refer to specifc sections without having to consult the entire chapter. In consequence, there is some repetition between the sections.

6.2 Grasses

The grasses listed in Table 4 are almost all perennial species, reproducing by seeds, rhizomes, stolons, tillers, and with sexually compatible species in the Netherlands.

Seeds can be destroyed by autoclaving, steaming, incinerating, composting and anaerobic fermenting (Table 5). In the latter 2 cases the temperature should be high enough and retained for a sufficiently long period (cf Keurcompost, sections 5.2.1 and 5.2.2). The seeds are rich in carbohydrates and are therefore suitable for ethanol production. Often the DDGS is further directed to feed production. However, as no GM grass species is authorised in the EU, fermenting plants may refuse to use these seed lots. Deep burial is also possible.

Once in the field, emerged seedlings may be tackled by hoeing. However, this does not result in 100% inactivation and may need to be repeated. Due to the formation of rhizomes and stolons mechanical weeding will be ineffective later on. Herbicide spraying may be the most economic method. Knowledge of the GM event will facilitate the choice of a herbicide.

Whenever the plants are flowering and setting seed, they can be mowed, collected and devitalised by composting, anaerobic fermentation, ethanol production, steaming or incineration. The stubble and underground parts can be killed spraying a systemic herbicide, once regrowth is sufficient for the plants to take up the chemical. Chemical treatment of the plants as a whole is possible as well, but may be less effective and will need a large dose of active ingredient. Spilled seeds can be allowed to germinate and sprayed with a herbicide.

Since grasses are cross-pollinating through wind neighbouring fields/meadows with sexually compatible species may be affected. In that case part of these fields have to be treated the same way although only for an area within isolation distance. As a guide for the isolation distance for grasses, Council Directive 66/401/EEC[41] may be consulted. However, it must be verified if flowering characteristics and outcrossing potential have changed in the GM event compared to the conventional crop.

Monitoring may be required, but may be difficult when the purpose was to renew/reseed grassland, and no other crop is available for rotation. If after a risk assessment monitoring is deemed necessary, the crop rotation may have to change to allow for volunteer monitoring.

[41] Council Directive 66/401/EEC of 14 June 1966 on the marketing of fodder plant seed. OJ 125, 11.7.1966, p. 2298–2308.

Table 5: Inactivation options for grasses

Plant developmental stage	Inactivation method	Remarks	Suitability
Seed	Composting	Temperature and retention time!	Reliability? Applying the waste hierarchy
	Fermentation Biogas production Ethanol production	 Temperature and retention time! DDGS is not suitable for feed (no feed authorisations)!	Reliability? Applying the waste hierarchy
	Steaming		Best results
	Autoclaving		Best results
	Incineration		Best results
	Deep burial		Not practical
Coated seed	**Incineration**		The only possibility
Seed sown, not emerged	Soil steaming		Reliability? Practicability?
	Mulching		Reliability? Too long treatment period
	Anaerobic soil disinfestation		Reliability? Too long treatment period
	Pre-emergence herbicides		Most practical
Vegetative phase	**Mechanical weeding**	Possible only for seedlings	Reliability? May need to be repeated
	Inundation		Not practical Too long treatment period
	Herbicides	Check tolerance of the commingling event(s)	Most practical
Flowering	**Mowing + herbicide** (crop residue: composting, anaerobic fermentation, ethanol production, steaming, incineration) The same for neighbouring areas with compatible species within isolation distance.	Check tolerance of the commingling event(s) DDGS is not suitable for feed (no feed authorisations)!	Most practical Applying the waste hierarchy
Seed set	**Mowing + herbicide** (crop residue: composting, anaerobic fermentation, ethanol production, steaming, incineration) The same for neighbouring areas with compatible species within isolation distance.	Check tolerance of the commingling event(s) DDGS is not suitable for feed (no feed authorisations)!	Most practical Applying the waste hierarchy
Spilled seed	Allow germination + **Herbicide treatment / Mechanical weeding**	Check tolerance of the commingling event(s)	Most practical

Plant developmental stage	Inactivation method	Remarks	Suitability
Aftercare	Monitoring for volunteers + inactivation	In case of potential presence non-germinated seeds (as sown; as produced during release)	

6.3 Winter hardy cereals

The cereals listed in Table 4 are all annual species, reproducing by seeds and tillers. They are predominantly self-pollinating although cross-pollination by wind is possible.

Seeds can be destroyed by autoclaving, steaming, incinerating, composting and anaerobic fermenting (Table 6). In the latter 2 cases the temperature should be high enough and retained for a sufficiently long period (cf Keurcompost, sections 5.2.1 and 5.2.2). The seeds are rich in carbohydrates and are therefore suitable for ethanol production. Often the DDGS is further directed to feed production. However, as no GM species of this group are authorised in the EU, fermenting plants may refuse to use these seed lots.

Once in the field, emerged seedlings may be tackled by hoeing. However, this does not result in 100% inactivation and may need to be repeated. Larger plants can be destroyed by disking and/or ploughing. Herbicide spraying may be the most economic method. Knowledge of the GM event will facilitate the choice of a herbicide.

Whenever the plants start to flower, they can be mowed, collected and devitalised by composting, anaerobic fermentation, steaming or incineration. The stubble and underground parts can be killed either by ploughing or by spraying a systemic herbicide, once regrowth is sufficient for the plants to take up the chemical. Chemical treatment of the plants as a whole is possible as well, but may be less effective and will need a large dose of active ingredient. Alternatively, the plants are allowed to set seed, as all species retain their seeds upon maturing. Seed-bearing plants can be harvested and the seeds and straw may be composted, fermented for biogas or ethanol production, steamed or incinerated. At that stage burning the field is another possibility. However, local regulations may prohibit this technique. Spilled seed can be either incorporated in the soil or allowed to germinate by establishing a false seedbed (Bond et al., 2007) followed by inactivation using herbicides or mechanical weeding. Ploughing is an option as cereal seed longevity in soil is rather short: after 1 year only a very small fraction may still be viable (Bond et al., 2007 and references therein).

Given the low potential of cross-pollination and expecting only a low amount of commingling seeds, outcrossing to neighbouring fields with the same species is probably not relevant. However, a decision can be taken only after a thourough risk assessment, provided that sufficient information on the commingling event is available, especially on outcrossing potential. Council Directive 66/402/EEC[42] does not set isolation distances for barley or wheat for seed production.

Monitoring for volunteers may not be necessary as these cereals fit into a crop rotation system where volunteers will be treated in the following crop. This is certainly the case when only minor amounts of the GM counterpart were present. Nevertheless, this consideration may need review when the GM cereal event is found in a different crop.

[42] Council Directive 66/402/EEC of 14 June 1966 on the marketing of cereal seed. OJ 125, 11.7.1966, p. 2309–2319

Table 6: Inactivation options for cereals (winter hardy)

Plant developmental stage	Inactivation method	Remarks	Suitability
Seed	Composting	Temperature and retention time!	Reliability? Apply waste hierarchy
	Fermentation Biogas production Ethanol production	 Temperature and retention time! DDGS not suitable for feed (no feed authorisations)!	Reliability? Apply waste hierarchy
	Steaming		Best results
	Autoclaving		Best results
	Incineration		Best results
	Deep burial		Not practical
Coated seed	**Incineration**		The only possibility
Seed sown, not emerged	Soil steaming		Reliability? Practicability?
	Mulching		Reliability? Too long treatment period
	Anaerobic soil disinfestation		Reliability? Too long treatment period
	Pre-emergence herbicides		Most practical
Vegetative phase	**Mechanical weeding**	Possible only for young plants	Reliability? May need to be repeated
	Disking and ploughing		
	Inundation		Not practical Too long treatment period
	Herbicides	Check tolerance of the commingling event(s)	Most practical
Flowering	**Mowing (harvesting) + disking, ploughing or herbicide treatment of the stubble** (collected crop residue: composting, anaerobic fermentation, ethanol production, steaming, incineration)	Check tolerance of the commingling event(s) DDGS is not suitable for feed (no feed authorisations)!	Applying the waste hierarchy.
	Chopping and ploughing		Most practical
Seed set	**Harvesting + disking, ploughing or herbicide treatment of the stubble** (seeds and straw: composting, anaerobic fermentation, ethanol production, steaming, incineration)	Check tolerance of the commingling event(s) DDGS is not suitable for feed (no feed authorisations)!	Most practical Applying the waste hierarchy
	Narrow windrow burning	Check local regulations	Probably not allowed
Spilled seed	**Ploughing**		Most practical

Plant developmental stage	Inactivation method	Remarks	Suitability
	Allow germination + **Herbicide treatment / Mechanical weeding**	Check tolerance of the commingling event(s)	
Aftercare	-		

6.4 Non-winter hardy cereals

In this category mainly maize is the important species in the Netherlands. Sorghum is gaining interest as a drought tolerant alternative for maize. Even rice cultivation is experimented at a very small scale. All are cultivated as annuals, and reproduce by seed. They are wind-aided cross-pollinators. Except for the crop no other sexually compatible species are present in the Netherlands.

Seeds can be destroyed by autoclaving, steaming, incinerating, composting and anaerobic fermenting (Table 7). In the latter 2 cases the temperature should be high enough and retained for a sufficiently long period (cf Keurcompost, sections 5.2.1 and 5.2.2). The seeds are rich in carbohydrates and are therefore suitable for ethanol production. Often the DDGS is further directed to feed production. Only in case the seed lot has been mixed with a GM maize event authorised in the EU, this option is open. If not known or not authorised, fermenting plants may refuse to use these seed lots. GM rice is not authorised in the EU. In the event of an authorised GM maize event, processing grains for food may be considered.

Pre-emergence treatments may not be succesful due to the depth maize is usually sown. For sorghum and rice these are valid options.

Once in the field, emerged seedlings may be tackled by hoeing. However, this does not result in 100% inactivation and may need to be repeated, as seedlings may be missed or seeds may germinate later. Larger plants can be destroyed by disking, chopping and/or ploughing. Herbicide spraying may be the most economic method. Knowledge of the GM event will facilitate the choice of a herbicide.

Whenever the plants are flowering and setting seed, they can be harvested (chopped), collected and devitalised by composting, anaerobic fermentation, ethanol production, steaming or incineration. If the GM maize event is authorised for feed use, the crop can be ensilaged as well. The stubble and underground parts will deteriorate over winter. Alternatively, the entire plants can be chopped and ploughed.

Because of the cross-pollination, part of neighbouring crop fields have to be treated the same way although only for an area within isolation distance. As a guide for the solation distance for maize, Council Directive 66/402/EEC[33] may be consulted. An isolation distance for rice is not mentioned. However, it must be verified if flowering characteristics and outcrossing potential have changed in the GM event compared to the conventional crop.

Spilled seed can be left on the field. Like the ploughed seeds, they will deteriorate in the next winter, because of the lack of dormancy, sensitivity to fungal disease and low temperatures. Conventional maize volunteers are seldom of agricultural significance in Europe and not at all in the Netherlands (VROM, 2007). Nevertheless these characteristics may have been altered in the GM event, necessitating inactivation of spilled seeds.

Maize is usually cultivated continuously making monitoring almost impossible. Monitoring may not be necessary given the frost sensitivity. However, frost sensitivity may have changed in the GM event. If after a risk assessment monitoring is deemed necessary, the crop rotation may have to change to allow for volunteer monitoring.

Table 7: Inactivation options for cereals (non-winter hardy)

Plant developmental stage	Inactivation method	Remarks	Suitability
Seed	Composting	Temperature and retention time!	Reliability? Applying the waste hierarchy.
	Fermentation Biogas production Ethanol production	Temperature and retention time! DDGS is suitable for feed, only if the GM maize event is authorised for feed	Reliability? Applying the waste hierarchy.
	Steaming		Best results
	Autoclaving		Best results
	Incineration		Best results
	Deep burial		Not practical
Coated seed	**Incineration**		The only possibility
Seed sown, not emerged	Soil steaming	Not for maize	Reliability? Practicability?
	Mulching	Not for maize	Reliability? Too long treatment period
	Anaerobic soil disinfestation		Reliability? Too long treatment period
	Pre-emergence herbicides	Not for maize	Most practical
Vegetative phase	**Mechanical weeding**	Possible only for young plants	Reliability? May need to be repeated
	Disking, chopping and ploughing		Most practical
	Inundation	Not for rice	Not practical Too long treatment period
	Herbicides	Check tolerance of the commingling event(s)	Most practical
Flowering	**Harvesting** (collected crop residue: composting, anaerobic fermentation, ethanol production, steaming, incineration, ensilage for feed use) The same for neighbouring fields with the same crop within isolation distance.	DDGS and ensilage is suitable for feed, only if the GM maize event is authorised for feed	Applying the waste hierarchy
	Chopping and ploughing The same for neighbouring fields with the same crop within isolation distance.		Most practical
	Wait for seeds to mature to inactivate		

Plant developmental stage	Inactivation method	Remarks	Suitability
Seed set	**Harvesting** (collected crop residue: composting, anaerobic fermentation, ethanol production, steaming, incineration, ensilage for feed use) The same for neighbouring fields with the same crop within isolation distance.	Temperature and retention time! DDGS and ensilage is suitable for feed, only if the GM maize event is authorised for feed	Applying the waste hierarchy
	Chopping and ploughing The same for neighbouring fields with the same crop within isolation distance.		Most practical
	Narrow windrow burning	Check local regulations	Probably not allowed
Spilled seed	-		
Aftercare	-		

6.5 Crucifers and species with similar characteristics

The most notorious crop within this group is oilseed rape, because of its capacity to shatter seeds, potential for outbreeding and development of secondary dormancy. Most crucifers are self-pollinating as well as cross-pollinating. Both wind and insects serve as pollen vectors. Next to the oilseed rape crop, other sexually compatible species are present in the Netherlands:

- *Brassica nigra,*
- *Brassica oleracea,*
- *Brassica rapa,*
- *Brassica carinata,*
- *Brassica juncea*
- *Raphanus raphanistrum,*
- *Hirschfeldia incana,*
- *Sinapis arvensis.*

For *Arabidopsis thaliana*, *Crambe* sp. and *Linum usitatissimum* no intra- or intergeneric hybrids are known within the genus (Beringen and Odé, 2016). For *Camelina sativa* no viable seeds are formed in crossings with other species in the Netherlands. *Carthamus tinctorius* can interbreed with *C. lanatus* (OGTR, 2019). *Lepidium campestre* has several relatives in the Netherlands and can hybridise with *L. heterophyllum* (Eriksson, 2009).

Seeds can be destroyed by autoclaving, steaming, incinerating, composting and anaerobic fermenting (Table 8). In the latter 2 cases the temperature should be high enough and retained for a sufficiently long period (cf Keurcompost, sections 5.2.1 and 5.2.2). The seeds are rich in fatty acids and are therefore suitable for biodiesel production. The by-product meal is a valuable component of animal feed. Only in case the seed lot has been mixed with a GM oilseed rape event authorised in the EU, this option is open. If not known or not authorised, crushing plants may refuse to use these seed lots. Deep burial is not an option, as it will induce secondary dormancy (except for *Carthamus tinctorius*). In the event of an authorised GM oilseed rape event, processing grains for food may be considered.

Seedlings and plants up to the 2 to 4-leaf stage can be inactivated using mechanical weeding equipment. Sometimes several successive operations are needed. Later vegetative stages may be disked or chopped. Herbicide spraying is another option. Knowledge of the GM event will facilitate the choice of a herbicide.

Vegetable brassicas are first sown indoors in growing medium (e.g. press pots) to be planted in soil later. At that stage seedlings and young plants can be collected and inactivated by composting, steaming, autoclaving or incineration.

In the bolting stage mowing is less suitable, as secondary sprouts will emerge from lower nodes. Disking or chopping is more effective. Chemical treatment of the plants as a whole is possible as well, but may be less effective and will need a large dose of active ingredient. Flowers open gradually from the top of the inflorescence to the bottom. Seeds develop in a similar way resulting in an overlap of flowering and seed set stage. The crop has to be harvested timely to prevent seed shattering, i.e. before complete maturity. Nevertheless, harvesting operations will inevitably disperse seeds in the field. Due to the potential for secondary dormancy induction, ploughing is not allowed. Instead, a false seedbed has to be prepared immediately after harvesting. Good germination conditions will allow most fallen seeds to germinate before winter and before soil preparation for the next crop. Seedlings can be devitalised spraying a suitable herbicide or by shallow cultivation.

In case sexually compatible species are present within isolation distance of the affected flowering crop, these need to be inactivated as well, as outcrossing cannot be prevented. However, not all wild relatives flower at the same time as the crop. As a guide for the isolation distance for oilseed rape, other brassicas and safflower, Council Directives 2002/57/EC[43] and 2002/55/EC [44] may be consulted. However, it must be verified if flowering characteristics and outcrossing potential have changed in the GM event compared to the conventional crop.

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[43] Council Directive 2002/57/EC of 13 June 2002 on the marketing of seed of oil and fibre plants. OJ L 193, 20.7.2002, p. 74–97.
[44] Council Directive 2002/55/EC of 13 June 2002 on the marketing of vegetable seed. OJ L 193, 20.7.2002, p. 33–59.

Monitoring may be necessary. However, as put forward by COGEM in the case of commingling with GT73, monitoring will have little value in the event oilseed rape was included earlier in the crop rotation, especially with low level presence of the GM event (COGEM, 2019). This GM event is authorised for food, feed and processing and environmental risks were found to be negligible.

Brassica volunteers in the next crop are usually inactivated before the 5-leaf stage, either mechanically or chemically (herbicide). Inactivation has to be done timely. As an example, oilseed rape volunteers in a succeeding sugar beet crop can show emergency flowering ("*noodbloei*") potentially exacerbating the problem (pers. comm. Ir. N. De Schrijver). Due to the developing sugar beet leaves the volunteer oilseed rape becomes stressed (competition for light) and will start flowering. If not timely devitalised, seeds will be formed and extend the volunteer problem.

Table 8: Inactivation options for crucifers

Plant developmental stage	Inactivation method	Remarks	Suitability
Seed	Composting	Temperature and retention time!	Reliability? Applying the waste hierarchy
	Fermentation Biogas production	Temperature and retention time!	Reliability? Applying the waste hierarchy
	Biodiesel production	Meal is suitable for feed, only if the GM oilseed rape event is authorised for feed	Applying the waste hierarchy
	Steaming		Best results
	Autoclaving		Best results
	Incineration		Best results
Coated seed	**Incineration**		The only possibility
Seed sown, not emerged	Soil steaming		Reliability? Practicability?
	Mulching		Reliability? Too long treatment period
	Anaerobic soil disinfestation		Reliability? Too long treatment period
	Soil solarisation	Limited applicability	Reliability? Too long treatment period
	Pre-emergence herbicides		Most practical
Seeds sown, seedlings and young plants in growing medium	Composting	Separation non-organic and organic material!	Reliability? Applying the waste hierarchy
	Steaming		Best results
	Autoclaving		Best results
	Incineration		Best results
Seedling and rosette stage	**Mechanical weeding**	Possible only for young plants	Reliability? May need to be repeated
	Disking, chopping		Most practical
	Heat weeding		
	Herbicides	Check tolerance of the commingling event(s)	Most practical
Bolting stage	Disking, chopping		
	Herbicides	Check tolerance of the commingling event(s)	Most practical

Plant developmental stage	Inactivation method	Remarks	Suitability
Flowering and Seed set	**Harvesting**: Seeds: *see first line* Crop residue: composting, anaerobic fermentation, ethanol production, steaming, incineration The same for neighbouring areas with compatible species within isolation distance.	No swathing, no herbicides! Avoid shattering (timely harvesting!) Temperature and retention time! DDGS is suitable for feed, only if the GM oilseed rape event is authorised for feed	Applying the waste hierarchy
	Narrow windrow burning	Check local regulations	Probably not allowed
Spilled seed	Prepare false seedbed Allow germination **Herbicide treatment / Mechanical weeding**	No ploughing! Check tolerance of the commingling event(s)	Mandatory
Aftercare	Monitoring for volunteers + inactivation	In case of potential presence non-germinated seeds (as sown; as produced during release)	

6.6 Annual legumes and species with similar characteristics

The annual legumes in Table 4 are all self-pollinating species, often cleistogamous, and are frost-sensitive, except for *Lupinus angustifolius* (narrow-leafed lupin). Peas at young stage tolerate mild frosts. Sunflower is self- and cross-pollinating with insects as a vector.

Seeds can be destroyed by autoclaving, steaming, incinerating, composting and anaerobic fermenting (Table 9). In the latter 2 cases the temperature should be high enough and retained for a sufficiently long period (cf Keurcompost, sections 5.2.1 and 5.2.2). Legume seeds are rich in proteins and are therefore suitable for animal feed. Oil in soya bean can be used for biodiesel production. The by-product meal is a valuable component of animal feed. Ethanol production is another option. Only in case the seed lot has been mixed with a GM soya bean event authorised in the EU, these options are open. If not known or not authorised, crushing plants and ethanol production sites may refuse to use these seed lots and feed producers are not allowed to use them. In the event of an authorised GM soya bean event, processing grains for food may be considered.

Pre-emergence treatments may not be succesful due to the depth the large seeds are usually sown.

Seedlings and plants up to the 2 to 4-leaf stage can be inactivated using mechanical weeding equipment. Sometimes several successive operations are needed. Later vegetative stages may be disked, chopped and/or ploughed. Mowing, eliminating the growing point, is another option for plants that start to flower. Repeated mowing may be necessary. Herbicide spraying is another option. Knowledge of the GM event will facilitate the choice of a herbicide.

Whenever the plants start to flower, they can be mowed, or disked, chopped and ploughed. Ensilaging is possible in case the intermixed GM soya bean event is authorised in the EU. Seed-bearing plants can be harvested and the seeds and straw may be composted, fermented for biogas production, biodiesel production, steamed or incinerated. Chemical treatment of the plants as a whole is possible as well, but may be less effective and will need a large dose of active ingredient. At that stage burning the field is another possibility. However, local regulations may prohibit this technique.

Because of sunflower cross-pollination, part of neighbouring areas with sunflower have to be treated the same way although only for an area within isolation distance. As a guide for the isolation distance for sunflower, Council Directive 2002/57/EC[42] may be consulted. However, it must be verified if flowering characteristics and outcrossing potential have changed in the GM event compared to the conventional crop.

Spilled seed can be left on the field. Like the ploughed seeds, they will deteriorate in the next winter. An exception is *Lupinus angustifolius*. The seeds of the wild types are hard-coated and frost tolerant, and they can remain viable in the soil for up to 20 years. However, most white-flowered cultivated species are soft-seeded with little dormancy (Boersma et al., 2007).

Monitoring for volunteers may not be necessary given the frost sensitivity (except for *Lupinus angustifolius*). However, it must be verified if frost sensitivity has changed in the GM event and monitoring may be necessary (risk assessment).

Table 9: Inactivation options for legumes (annual)

Plant developmental stage	Inactivation method	Remarks	Suitability
Seed	Composting	Temperature and retention time!	Reliability? Applying the waste hierarchy
	Fermentation Biogas production Ethanol production	Temperature and retention time! DDGS is not suitable for feed, unless the GM soya bean event is authorised for feed	Reliability? Applying the waste hierarchy
	Biodiesel production	Meal is suitable for feed, only if the GM soya bean event is authorised for feed	Applying the waste hierarchy
	Use in feed	Only if the GM soya bean event is authorised for feed	Applying the waste hierarchy
	Steaming		Best results
	Autoclaving		Best results
	Incineration		Best results
	Deep burial		Not practical
Coated seed	**Incineration**		The only possibility
Seed sown, not emerged	Anaerobic soil disinfestation		Reliability? Too long treatment period
Vegetative phase	**Mechanical weeding**		Reliability? May need to be repeated
	Heat weeding		
	Disking, chopping and ploughing		Most practical
	Mowing		Most practical
	Mowing + ensilaging (soya bean)	Only when the GM soya bean event is authorised for feed	Applying the waste hierarchy
	Inundation		Too long treatment period
	Herbicides	Check tolerance of the commingling event(s)	Most practical
Flowering	**Disking, chopping and ploughing**		Most practical
	Mowing		Most practical
	Mowing + Ensilaging (soya bean)	Only when the GM soya bean event is authorised for feed	Applying the waste hierarchy
	Herbicides		Most practical
	Sunflower: the same for neighbouring areas with sunflower within isolation distance		

Plant developmental stage	Inactivation method	Remarks	Suitability
Seed set	**Harvesting** (collected seeds and straw: composting, anaerobic fermentation, biodiesel production, steaming, incineration, ensilage for feed use)	Temperature and retention time! DDGS, meal and ensilage are suitable for feed, only if the GM soya bean event is authorised for feed	Applying the waste hierarchy
	Chopping and ploughing	Not for *Lupinus angustifolius* (narrow-leafed lupin)!	Most practical
	Narrow windrow burning	Check local regulations	Probably not allowed
	Sunflower: the same for neighbouring areas with sunflower within isolation distance		
Spilled seed	-	For *Lupinus angustifolius*: allow germination + Herbicides/Mechanical weeding	
Aftercare	-	Monitoring + inactivation is possibly necessary for *Lupinus angustifolius*	

6.7 Perennial legumes

Both alfalfa and white clover multiply by seeds and stolons, are frost tolerant, cross-pollinate with the help of insects and have sexually compatible species growing in the Netherlands.

Alfalfa is mostly grown in monoculture, white clover is often mixed with grasses for temporal or permanent meadows.

Seeds can be destroyed by composting and anaerobic fermenting (Table 10). In both cases the temperature should be high enough and retained for a sufficiently long period (cf Keurcompost, sections 5.2.1 and 5.2.2). Although legumes are valued for their high protein content, the seeds also contain carbohydrates that can be turned into ethanol. Often the DDGS is further directed to feed production. However, as no GM alfalfa or white clover is authorised in the EU, fermenting plants may refuse to use these seed lots. As with other species steaming, autoclaving, incineration and deep burial are also possible.

Once in the field, emerged seedlings may be tackled by hoeing. However, this does not result in 100% inactivation and may need to be repeated. Due to the formation of stolons mechanical weeding will be less effective later on, unless a rotary tooth cultivator or a rotary tiller-type machine is used. Herbicide spraying may be the most economic method. Knowledge of the GM event will facilitate the choice of a herbicide.

Whenever the plants are flowering and setting seed, they can be mowed, collected and devitalised by composting, anaerobic fermentation, ethanol production, steaming or incineration. The stubble and underground parts can be killed spraying a systemic herbicide, once regrowth is sufficient for the plants to be treated. Chemical treatment of the plants as a whole is possible as well, but may be less effective and will need a large dose of active ingredient. Spilled seeds can be allowed to germinate and sprayed after emergence.

Since alfalfa and white clover are cross-pollinating through wind, neighbouring fields/areas with the same crop or other sexually compatible species may be affected. In that case part of these fields have to be treated the same way although only for an area within isolation distance. As a guide for the isolation distance for these crops, Council Directive 66/401/EEC[32] may be consulted. However, it must be verified if flowering characteristics and outcrossing potential have changed in the GM event compared to the conventional crop.

Monitoring may be required, but may be difficult when the purpose was to renew meadows, and no other crop is available for rotation. If after a risk assessment monitoring is deemed necessary, the crop rotation may have to change to allow for volunteer monitoring (e.g. including a fallow period).

Table 10: Inactivation options for legumes (perennial)

Plant developmental stage	Inactivation method	Remarks	Suitability
Seed	Composting	Temperature and retention time!	Reliability? Applying the waste hierarchy
	Fermentation Biogas production Ethanol production	Temperature and retention time! DDGS is not suitable for feed (no feed authorisations)!	Reliability? Applying the waste hierarchy
	Steaming		Best results
	Autoclaving		Best results
	Incineration		Best results
	Deep burial		Not practical
Coated seed	**Incineration**		The only possibility
Seed sown, not emerged	Soil steaming		
	Mulching		Reliability? Takes too long
	Anaerobic soil disinfestation		Reliability? Takes too long
	Pre-emergence herbicides		Most practical
Vegetative phase	**Mechanical weeding**	Possible only for seedlings	Reliability? May need to be repeated
	Inundation		Too long treatment period
	Herbicides	Check tolerance of the commingling event(s)	Most practical
Flowering	**Mowing + herbicide** (crop residue: composting, anaerobic fermentation, ethanol production, steaming, incineration)	Check tolerance of the commingling event(s) DDGS is not suitable for feed (no feed authorisations)!	Applying the waste hierarchy
	Herbicides		Most practical
	The same for neighbouring areas with compatible species within isolation distance.		
Seed set	**Mowing + herbicide** (crop residue: composting, anaerobic fermentation, ethanol production, steaming, incineration) The same for neighbouring areas with compatible species within isolation distance.	Check tolerance of the commingling event(s) Temperature and retention time! DDGS is not suitable for feed (no feed authorisations)!	Most practical Applying the waste hierarchy
Spilled seed	Allow germination + **Herbicides / Mechanical weeding**	Check tolerance of the commingling event(s)	Most practical
Aftercare	Monitoring + inactivation	In case of flowering and seed set	

6.8 Tuber crops

The typical tuber crop is potato. Today, sweet potato is cultivated as an annual on a small scale but is gaining interest. Tubers (seed potatoes) and slips (small rooted pieces of the sweet potato tuber) are used to start a new crop. Seeds are formed depending on the cultivar and have their value in breeding programmes. The vegetative parts are frost sensitive.
Botanically speaking potatoes are stem-tubers (enlarged stolons), whereas sweet potatoes are root-tubers. They are nevertheless discussed here together because of the similar characteristics in relation to this report. Also, neither is sold as seed.

Tubers for planting can be devitalised by composting, fermenting, freezing, steaming, autoclaving or incineration (Table 11). The tubers are rich in carbohydrates and are therefore suitable for ethanol production. Often the DDGS is further directed to feed production. However, as no GM potato is authorised in the EU, fermenting plants may refuse to use these tubers.

Once planted it is not feasable to uproot seed potatoes mechanically because of the small size. Pre-emergence herbicides will not reach them. Only inundation will inactivate at this stage.

In the vegetative stage the plants can be inactivated using systemic herbicides. Knowledge of the GM event will facilitate the choice of a herbicide. Mechanical weeding, physical weeding, disking, chopping and ploughing or mowing are not or less suitable as the tubers or sliced tubers will re-sprout. Consequently, mechanical (harsh) weeding needs to be repeated. Further on in the season the foliage may be treated as is practised just before harvest. Flail mowers are most suitable for mechanical haulm removal possibly combined with flaming. A traditional method uses a chemical desiccant (herbicide) treatment. Potatoes are then harvested and subsequently devitalised the same way as seed potatoes. To reduce the number left behind in the soil, the settings of potato harvester and travelling speed need to be optimised.

Potato seed production in fields varies with cultivar and weather conditions. Tetraploid *S. tuberosum* is self-compatible. Cross-pollination may happen with plants in neighbouring fields. Sweet potatoes rarely flower when the daylight is longer than 11 hours.

As not all tubers will be dug up, monitoring for volunteers in the next crop is required, followed by inactivation. Also, volunteers originating from seeds are possible and may be a more important group of volunteers compared to volunteers from tubers left behind, especially after a mild winter. Once shed, potato seeds can remain viable in soil for 3-9 years. However, normal farming practices already include inactivation of volunteers in relation to the mandatory control of *Phytophthora infestans* in the succeeding crops. Consequently, potato volunteers are already effectively inactivated.

Table 11: Inactivation options for tuber crops

Plant developmental stage	Inactivation method	Remarks	Suitability
Tubers	Composting		Applying the waste hierarchy
	Fermentation Biogas production Ethanol production	 DDGS is not suitable for feed (no feed authorisations)!	Reliability? Applying the waste hierarchy
	Freezing		Best results
	Steaming		Best results
	Autoclaving		Best results
	Incineration		Best results
Tubers planted, not emerged	Inundation		Too long treatment period
	Wait until plants emerge to inactivate		
Vegetative phase	**Herbicides**	Check tolerance of the commingling event(s)	Most practical
	Mechanical (harsh) weeding	May need a 2nd passage	
Flowering	Flail mowing, flailing, or Flaming, burning, or Chemical desiccant (herbicide) + **Harvesting** tubers Tubers: *see first line* The same for neighbouring areas with potatoes within isolation distance	May need a 2nd passage	Most practical
Seed set	Flail mowing, flailing, or Flaming, burning, or Chemical desiccant (herbicide) + **Harvesting** tubers Tubers: *see first line* The same for neighbouring areas with potatoes within isolation distance	May need a 2nd passage	Most practical
Maturing (tubers developed)	Flail mowing, flailing, or Flaming, burning, or Chemical desiccant (herbicide) + **Harvesting** tubers Tubers: *see first line*	May need a 2nd passage Machine harvesting settings!	Most practical
Aftercare	Monitoring for volunteers + inactivation		Already general practice

6.9 Root crops

Root crops typically are harvested in the first year, but, when left in the soil, flower in the second year (biennial). In certain conditions and depending on the variety bolting occurs already in the first year. The vegetative parts including the roots of sugar beet, chicory and carrot are sensitive to freezing. Seeds of sugar beet and chicory are able to develop secondary dormancy in hostile conditions.

Seeds can be destroyed by composting and anaerobic fermenting (Table 12). In both cases the temperature should be high enough and retained for a sufficiently long period (cf Keurcompost, sections 5.2.1 and 5.2.2). Steaming, autoclaving and incinerationare also possible. Deep burial is not an option, as it will induce secondary dormancy (except for *Daucus carota*).

Small, young plants can be mechanically removed up to the 4-leaf stage (BBCH-14 stage). However, this does not result in 100% inactivation and may need to be repeated. Furthermore, physical weeding, inundation and spraying with herbicides is possible. Knowledge of the GM event will facilitate the choice of a herbicide. Disking, chopping and/or ploughing are not suitable as part of the plant, especially the crowns, are able to re-sprout. Mowing in the rosette phase is equally not an option as the growing point is at or near the soil surface. Bolting plants are usually manually removed from the field and allowed to desiccate.

At harvest the leaves are left in the field and the roots are collected. Roots (and crowns) can be devitalised by composting, fermenting, freezing, steaming, autoclaving or incineration. If crowns cannot be removed from the field they may be chopped and ploughed, as in case of roots left behind. Re-growth is still possible (groundkeepers) and should be treated in the next crop. The roots are rich in carbohydrates and are therefore suitable for ethanol production. Often the DDGS is further directed to feed production. Only in case the seed lot has been mixed with a GM sugar beet event authorised in the EU, this option is open. If not known or not authorised, fermenting plants may refuse to use these seed lots. Today, only one GM sugar beet event tolerant to glyphosate is approved for food and animal feed in the EU. In this case, processing roots for food (sugar) may be considered.

If seeds have been dispersed (bolters), the field should not be ploughed. Instead, a false seedbed has to be prepared immediately after harvesting. Good germination conditions will allow most fallen seeds to germinate before winter and before soil preparation for the next crop. Seedlings can be devitalised spraying a suitable herbicide or by shallow cultivation. In *Daucus carota* (carrot) dormancy is less of a problem.

Monitoring focusses on groundkeepers and seedlings. They may be inactivated in the same way weeds are managed in the succeeding crop.

Table 12: Inactivation options for root crops

Plant developmental stage	Inactivation method	Remarks	Suitability
Seed	Composting	Temperature and retention time!	Reliability? Applying the waste hierarchy
	Fermentation Biogas production	Temperature and retention time!	Reliability? Applying the waste hierarchy
	Steaming		Best results
	Autoclaving		Best results
	Incineration		Best results
Coated seed	**Incineration**		The only possibility
Seed sown, not emerged	Soil steaming		
	Mulching		Too long treatment period
	Pre-emergence herbicides		Most practical
Vegetative phase	**Mechanical weeding**	Possible only for seedlings	Reliability? May need to be repeated
	Heat weeding		
	Inundation		Too long treatment period
	Herbicides	Check tolerance of the commingling event(s)	Most practical
Maturing (roots developed)	Harvesting Leaves: (chopping),and ploughing Roots and crowns: composting, fermenting, freezing, steaming, autoclaving or incineration	DDGS is suitable for feed, only if the GM sugar beet event is authorised for feed	Applying the waste hierarchy
	Herbicide + chopping and ploughing after 1 month	Check tolerance of the commingling event(s)	Most practical
Flowering	**Manual pulling + desiccation**		Already general practice
Seed set	**Manual pulling + incineration**	Avoid seed dispersal! (bagging)	Most practical
Spilled seed	In case of bolters with seeds: Prepare false seedbed Allow germination Herbicide treatment or mechanical weeding	No ploughing! Check tolerance of the commingling event(s)	
Aftercare	Monitoring for volunteers + inactivation	Re-growth from crowns/roots and seedlings	

6.10 Other annual fruit & vegetable species (non-winter hardy)

In Sections 6.5, 6.6 and 6.9 some vegetables have been covered. This group deals with other vegetables and annual fruit species. It comprises species for protected cultivation or species that are grown outdoors after the last spring frost. None of them can withstand freezing. No sexually compatible species are present in the Netherlands, except for lettuce.

Seed lots can be inactivated in the same way as described above for other species, i.e. via composting, fermentation, steaming, autoclaving, incineration and deep burial (Table 13). For the first 2 the temperature should be high enough and retained for a sufficiently long period (cf Keurcompost, sections 5.2.1 and 5.2.2). Seeds are sown indoors in growing medium (e.g. press pots, plugs) to be planted later in growbags, hydroponics, soil, etc. At that stage seedlings and young plants can be inactivated by composting, steaming, autoclaving or incineration. Alternatively, spreading on agricultural land and plowing is as effective.

In soil-bound culture plants can be devitalised by mowing, disking, chopping and ploughing using mechanical weeding machinery, cultivators, etc. In soilless culture, plants need to be collected first to be treated. Suitable methods are composting, steaming, autoclaving or incineration and incorporation in soil.

Also flowering and fruit-bearing plants may be treated the same way. Cross-pollination in protected cultivation is of a lesser concern. In greenhouses equiped with insect screens, as is often the case, these will prevent pollination by insects beyond the greenhouse. Pumpkins and squashes are usually grown outdoors and are visited by insects that may pollinate neighbouring crops. If present within isolation distance of the affected crop, these need to be inactivated as well. As a guide for the isolation distance for vegetables, Council Directive 2002/55/EC[35] may be consulted. However, it must be verified if flowering characteristics and outcrossing potential have changed in the GM event compared to the conventional crop.
Lettuce, a self-pollinating species is harvested before bolting.

No monitoring is deemed necessary as all plant material incorporated into the soil will deteriorate in winter.

Table 13: Inactivation options for fruit and vegetables

Plant developmental stage	Inactivation method	Remarks	Suitability
Seed	Composting	Temperature and retention time!	Reliability? Applying the waste hierarchy
	Fermentation Biogas production	Temperature and retention time!	Reliability? Applying the waste hierarchy
	Steaming		Best results
	Autoclaving		Best results
	Incineration		Best results
	Deep burial		Not parctical
Coated seed	**Incineration**		The only possibility
Seeds sown, seedlings and young plants in growing medium	Composting	Separation non-organic and organic material!	Applying the waste hierarchy
	Steaming		Best results
	Autoclaving		Best results
	Incineration		Best results
	Incorporation in soil	Separation non-organic and organic material!	Land availability?
Soil-bound culture (vegetative phase)	**Mowing/disking/chopping + ploughing**		Most practical. May need to be repeated
	Herbicides	Check tolerance of the commingling event(s)	Most practical
Soilless culture (vegetative and generative phase)	Composting	Separation non-organic and organic material! Temperature and retention time!	Reliability? Applying the waste hierarchy
	Incorporation in soil	Separation non-organic and organic material!	Land availability?
	Steaming		Best results
	Autoclaving		Best results
	Incineration		Best results
Soil-bound culture (generative phase)	**Mowing/disking/chopping + ploughing** The same for neighbouring fields with compatible crops within isolation distance.		
Aftercare	-		

6.11 Bulbs

Onions and floral species, both winter hardy and frost sensitive are taken together in this section.

Onions are either sown closely spaced in the first year, uprooted and planted in the second year to be harvested as a bulb in early summer, or, sowing and harvesting, in late summer, occur in the same year. Flowering in onion is only desired in breeding programmes. Floral bulb species, on the contrary, are cultivated for their flowers. Bulbs are marketed for planting in public and private gardens, as cut flowers and bulbs in pot. In order to flower they need a vernalisation treatment, in soil or in specially equiped facilities. The non-winter hardy species need to be collected from soil, stored in frost-free conditions to be planted again the next season.

Starting from a non-GM bulb, vegetatively multiplied progeny cannot become mixed with GM bulbs. However, in trade and retail they may be commingled with other material.

Seed lots, bulbils ("*broedbolletjes*"), cormels, scales, cuttings of rhizomes can be inactivated the same way as described above for other species, i.e. via composting, fermentation, steaming, autoclaving, incineration and deep burial (Table 14). For seeds the temperature should be high enough and retained for a sufficiently long period (cf Keurcompost, sections 5.2.1 and 5.2.2).

With foliage developed above ground a systemic herbicide treatment is the most effective. Early harvesting (lifting the whole plant) is another possibility.
For comparison, the difficult to control weed *Cyperus* spp. forms bulbs, rhizomes and tubers. Proposed methods to inactivate are extensive mowing depleting nutrients, repeated tillage operations uprooting the rhizomes and tubers to dessicate, and soil solarisation (Riemens et al., 2008).

Bulbs kept indoors (in the forcing period) and further grown in greenhouses can be collected and composted, steamed, autoclaved and incinerated. Incorporation in soil is another possibility provided that this is deep enough to prevent regrowth, potentially after shredding.

Onions are harvested before flowering. In cut flower production flowers are harvested before opening ("as soon as the plants show colour") and therefore pollination and seed setting are prevented. Likewise, pot plants are distributed before flowers open. Outdoor flowering bulbs may pollinate plants in neighbouring fields. However, seed set and germination is usually not an issue, as flowers are topped for the plant to put all growing energy in the bulb. Monitoring can be limited to regrowth of soil incorporated material.

Table 14: Inactivation options for bulbs

Plant developmental stage	Inactivation method	Remarks	Suitability
Seed, bulbs, bulbils, cuttings	Composting	Temperature and retention time!	Reliability? Applying the waste hierarchy
	Fermentation Biogas production	Temperature and retention time!	Reliability? Applying the waste hierarchy
	Steaming		Best results
	Autoclaving		Best results
	Incineration		Best results
	Deep burial		Not practical
Coated seed	**Incineration**		The only possibility
Seed sown, not emerged	Soil steaming		
	Mulching		Too long treatment period
	Biological soil disinfestation		Too long treatment period
	Pre-emergence herbicides		Most practical
Bulbs, bulbils, etc. planted, not emerged (soil)	Lifting and inactivation: bulbs: *see first line*	Recovery rate?	Small bulbils, etc. may escape
	Tillage (e.g. rotary cultivator)		Small bulbils, etc. may escape
	Inundation		Too long treatment period
Vegetative and generative phase (soil)	Lifting/harvest and inactivation: bulbs: *see first line*	Recovery rate?	
	Herbicides	Check tolerance of the commingling event(s)	Most practical
Bulbs planted in pots, bulbfust ("broeifust"), etc. (forcing period) Vegetative and generative phase	Composting	Separation non-organic and organic material! Temperature and retention time!	Reliability? Applying the waste hierarchy
	Incorporation in soil	Separation non-organic and organic material! Deep!	Land availability?
	Steaming		Best results
	Autoclaving		Best results
	Incineration		Best results
Aftercare	Monitoring for regrowth of soil incorporated material + inactivation		

6.12 Pot and bedding plants

Pot and bedding plants are either obtained starting from seeds or via vegetative propagation. Starting from a non-GM mother plant, vegetatively multiplied progeny cannot become mixed with GM material. However, in trade and retail they may be commingled with other material.

Starting material can be inactivated the same way as described above for other species, i.e. via composting, fermentation, steaming, autoclaving, incineration and deep burial (Table 15). For the first 2 the temperature should be high enough and retained for a sufficiently long period (cf Keurcompost, sections 5.2.1 and 5.2.2).

Plants are mostly grown in greenhouses before sale, except for pot chrysanths. In case a commingling is reported, they can be collected and composted, steamed, incorporated in soil, autoclaved and incinerated.

Plants already in public and private gardens (vegetative and generative) may be uprooted and composted. Herbicide treatment is another possibility, but will most likely be avoided in public and private gardens. Most of the pot and bedding plants listed in Table 4 are frost sensitive. As a consequence, seed set and volunteers will be a minor problem.

6.13 Trees

Trees may be propagated in different ways. Grafting on rootstocks is often used for fruit trees. Only the rootstocks are multiplied by seed. Trees for wood production or ornamental purposes are multiplied by stem cuttings (e.g. *Populus* spp.), root cuttings, etc., or seed. Starting from a non-GM tree, vegetatively multiplied progeny cannot become mixed with a GM tree. However, in trade and retail they may be commingled with other material.

In the nurseries the young plants are either grown in plugs or pots (protected), or they are kept in soil. Still small and in pots they can be collected and composted, steamed, incorporated in soil, autoclaved and incinerated (Table 16). Larger plants can be shredded first and composted or used for fermentation to turn into biogas or ethanol. Woodchips can be used in private and public gardens as mulching material.

Unless the commingling is noticed years after planting, cross-pollination, seed set and sprouting of roots is no issue with trees.

6.14 Non-classified species

New species and those not described in any of the above listed groups, can be assessed taking into account the characteristics as mentioned in Section 2.3 and Table 4. If no information on the biology can be obtained or the situation urges for quick action, the most drastic inactivation methods can be applied (e.g. incineration).

Table 15: Inactivation options for pot and bedding plant

Plant developmental stage	Inactivation method	Remarks	Suitability
Seed, vegetative starting material	Composting	Temperature and retention time!	Reliability? Applying the waste hierarchy
	Fermentation Biogas production	Temperature and retention time!	Reliability? Applying the waste hierarchy
	Steaming		Best results
	Autoclaving		Best results
	Incineration		Best results
	Deep burial		Not practical
Coated seed	**Incineration**		The only possibility
Plants in pots (vegetative and generative phase)	Composting	Separation non-organic and organic material! Temperature and retention time!	Applying the waste hierarchy
	Fermentation Biogas production	Separation non-organic and organic material! Temperature and retention time!	Reliability? Applying the waste hierarchy
	Incorporation in soil	Separation non-organic and organic material!	Land availability?
	Steaming		Best results
	Autoclaving		Best results
	Incineration		Best results
Plants in soil (vegetative and generative phase)	Uprooting and composting	Temperature and retention time!	Applying the waste hierarchy

Table 16: Inactivation options for trees

Plant developmental stage	Inactivation method	Remarks	Suitability
Young plants in nurseries	Composting	Separation non-organic and organic material!	Applying the waste hierarchy
	Steaming		Best results
	Incorporated in soil	Separation non-organic and organic material!	
	Autoclaving		Best results
	Incineration		Best results
	Fermentation Biogas production Ethanol production		Applying the waste hierarchy
Large plants	Steaming		In case a high-capacity installation is available
	Schredding + Composting		Applying the waste hierarchy
	Schredding + Fermentation Biogas production Ethanol production		Applying the waste hierarchy
	Shredding into woodchips for use in gardens		Applying the waste hierarchy
	Tree stumps: grinding		

7 Conclusions

This study was set up to develop guidelines for the inactivation of plants and seeds that unintentionally became commingled with non-authorised GM events.

An inventory of species for which GM events are approved for commercial release and/or trialled in the field in at least one country confirmed the broad botanical range with nearly 160 species. A selection of species that are relevant for the Netherlands were further classified based on biology documents, scientific literature and reports. This resulted in 13 categories.

At the same time, inactivation methods were identified and described with a specific focus on applicability, advantages and shortcomings. They were grouped according to the primary mode of action (physical treatment, biological treatment, mechanical treatment or chemical treatment). Since only few references are specific for GM plants, inactivation methods that are used to combat weeds in general were referred to where available. On the other hand, weeds are not completely comparable to domesticated crops as weeds may be more resilient. In consequence, GM crop species may be controlled with less stringent methods. Finally, scientific literature presents diverging efficacy figures on some methods which may be related to the effect of environmental conditions.

In a final chapter, an analysis is presented of the adequacy of the different methods for the different GM plant categories, linking information on the biology of the plants with the applicability and efficacy of the inactivation methods. A decision tree is proposed as well as summary tables that identify the most suitable methods depending on the type of material and/or stage of development.

Notwithstanding the specific indications per category, the following conclusions can be drawn:

- With the exception of autoclaving and incineration, no method inactivates seeds and plants for 100%.
- Seeds of all species can be treated (almost) the same way: incineration is always adequate, next to steaming and autoclaving. For tuber crops, freezing is an additional valid option.
- Wet biomass can always be composted or fermented, but care must be taken when the material (potentially) contains seeds. In that case it is important to maintain the required high temperature for a sufficiently long period of time.
  Steaming is also a feasible method, in particular when other material than biomass (pots, soil, etc.) is present.
- In the field, the use of a suitable herbicide to inactivate a crop is the most practical option, but the choice of the herbicide must take into account that the GM may have been modified to be tolerant to specific herbicides. When applied correctly (development stage, dose, weather conditions), it will give a high inactivation efficiency. However, other methods are available when farmers chose not to use chemical plant protection products (farmers wishing to minimise the use of pesticides, organic farmers). Mechanical weeding is the most obvious alternative, next to ploughing (incorporation of the biomass into the soil). In view of the principles of recycling and recovery, the options of mowing or harvesting and collection of the seed and/or the crop residue, the options of composting, fermentation (biogas and ethanol production), biodiesel production and use as or in feed in case the commingling GM event is authorised to, are valuable alternatives.
- Inundation, soil steaming, solarisation, mulching and biological disinfestation of the soil remain options, but have disadvantages (e.g. long treatment period)and will usually be considered impractical.
- Whereas farmers will probably like to resume their business as soon as possible, they will choose for short duration inactivation methods, if left the choice. Often this will involve the use of herbicides with short carry-over effect or incorporation of the plant material into the soil, also because machinery is on hand. Mulching, anaerobic soil disinfestation, inundation, soil solarisation may result in missing a growing season.
- Shattered seeds and later-germinating seeds can be coped with establishing a false seedbed to allow germination followed by inactivation.
- The choice of the following crop should allow for monitoring and inactivation of volunteers. This may involve a change in the usual rotation plan.

In addition to the technical aspects of the inactivation techniques, some general observations were highlighted through the limited experience with previous cases and interactions with the persons involved:

- Identification and quantification of the commingled GM material is required to perform a risk assessment. If it concerns GM plants that have been authorised for import as viable material for processing and food and/or feed use in the EU, some of the environmental risks have already been determined. Although an environmental risk assessment will be a prerequisite for an authorisations for import of viable GM plant material, the scope may be significantly different: for import the focus will be on spillage during transportation, whereas commingling may result in the presence in farmers' fields in optimal cultivation conditions. The relevance of the evaluation for import will therefore need to be considered on a case-by-case basis. Nevertheless, the authorisation may allow other options (such as direction for animal feed).
- A key objective is to intervene before spatial dispersal (via pollen to compatible crops) or temporal dispersal (establishment in the seedbank) occurs. For crops that are seeded, such introduction will already occur as of sowing and there always remains a chance that some seeds did not germinate in the initial period.
- The applicability of certain methods is determined by the availability of expensive equipment and suitable facilities. An autoclave may be appropriate for inactivating small quantities, yet this may not be compatible with large batches of soiled material. Furthermore, certain types of installations (e.g. biodiesel or ethanol production plants) may be specialised in routine handling of certain materials and may not be open to receive an occasional diverging batch. In particular if this generates by-products for other sectors (e.g. feed) this may create issues for segregation of products related to authorisations and labelling.
- Ideally, inactivation occurs as close as possible to the site where the material is discovered. However, the most efficient inactivation methods may require transportation. During transportation care must be taken that no material is lost. The discrepancy between the indications of the ADR legislation and the *Regeling GGO* need to be sorted out in this respect (packaging instructions).
- Monitoring can be implemented to confirm the efficacy of the inactivation or provide a tracking system of remaining problems. Its usefulness may to be determined on a case-by-case basis taking into account the species, developmental stage, level of commingling, rotation history and whether the commingling GM event has been authorised for food, feed and processing.
- When GMO presence is detected that leads to an obligation to remove the material, in addition to identifying suitable methods, the responsible parties (including inspection), the type of verifications and the type of expected reporting can be indicated more explicitly. A documented process and follow-up would be useful to track compliance and to learn on best practices.

In spite of the large, global scale deployment of GM plants, the number of cases that have been reported and that required inactivation of plant material remains very limited. However, as more countries are introducing plant biotechnology applications and product authorisations continue to be unsynchronised, this situation is not expected to become less demanding. Furthermore, once an unapproved GMO presence is detected, the impact can be far reaching and fast action is required. As techniques develop, more adequate options may become available. The approach proposed in this study, justifying the choice of inactivation method on biological features of the GM species, provides a framework for future cases.

8 References

8.1 References on the biology of species

CABI Invasive Species Compendium: https://www.cabi.org/isc/

CFIA, Biology Documents - Companion Documents for Directive 94-08, available at: https://www.inspection.gc.ca/plant-health/plants-with-novel-traits/applicants/directive-94-08/biology-documents/eng/1330723572623/1330723704097

GEAC, Series of Crop Specific Biology Documents, available at: http://www.geacindia.gov.in/resource-documents.aspx

OECD, Crop biology documents, available at: https://www.oecd.org/env/ehs/biotrack/consensusdocumentsfortheworkonharmonisationofregulatoryoversightinbiotechnologybiologyofcrops.htm

OGTR, Crop biology documents, available at: http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/biology-documents-1

Wikipedia: https://en.wikipedia.org/

8.2 Other references

Ackroyd, V., Besançon, T., Bunchek, J., Cahoon, C., Chandran, R., Curran, W., Flessner, M., Klodd, A., Lingenfelter, D., Mirsky, S., Ryan, M., Sandy, D., VanGessel, M., Vollmer, K., and Ward, M. (2019). "A practical guide for integrated weed management in Mid-Atlantic grain crops."

Ascard, J. (1994). Dose–response models for flame weeding in relation to plant size and density. *Weed Research* **34**, 377-385.

Bashandy, H., and Teeri, T. H. (2017). Genetically engineered orange petunias on the market. *Planta* **246**, 277-280.

Beringen, R., and Odé, B. (2016). Herziening van kenmerken van planten ten behoeve van de lijst met inperkingsmaatregelen. CGM 2016-01. pp. 66.

Blok, W. J., Lamers, J. G., Termorshuizen, A. J., and Bollen, G. J. (2000). Control of Soilborne Plant Pathogens by Incorporating Fresh Organic Amendments Followed by Tarping. *Phytopathology™* **90**, 253-259.

Boersma, J. G., Buirchell, B. J., Sivasithamparam, K., and Yang, H. (2007). Development of a PCR marker tightly linked to mollis, the gene that controls seed dormancy in Lupinus angustifolius L. *Plant Breeding* **126**, 612-616.

Bollen, G. J., Derckx, J. M. H., Glas, J. J., Groenewegen, J. H., Kruyk, P. A., Meinders, H., Meijndert, J., Nienhuis, J. K., Runia, W. T., Sonneveld, C., and Verveer, J. B. (1981). Grondstomen. *Informatiereeks* **71**.

Bond, W., Davies, G., and Turner, R. (2007). The biology and non-chemical control of Volunteer Cereals. pp. 5. Henry Doubleday Research Association, the organic organisation.

COGEM (2001). Beproeving en vermeerdering van genetisch gemodificeerde aardappelplanten met een verlaagd amylosegehalte. pp. 9. CGM/011029-01.

COGEM (2015). Advies: Overleving en kruisbaarheid van Gossypium herbaceum in Nederland. pp. 6. CGM/150407-01.

COGEM (2017). Advies 'milieurisico's van niet-toegelaten transgene petunia's met oranje bloemen'. pp. 13. CGM/170522-04.

COGEM (2019). Advies procedure voor verdelging van niet-toegelaten genetisch gemodificeerde koolzaadplanten. pp. 3. CGM/190215-02.

de Kool, S. (2008). Inundatie in de bloembollenteelt. *In* "Telen met toekomst", pp. 4. WUR.

Di Gioia, F., Hong, J. C., Ozores-Hampton, M., Zhao, X., Wilson, C., Thomas, J., Li, Z., Pisani, C., Guo, H., Paudel, B., Albano, J., Butler, D. M., and Rosskopf, E. N. (2020). Anaerobic soil disinfestation: nutrient cycling and potential environmental impact. pp. 51-62. International Society for Horticultural Science (ISHS), Leuven, Belgium.

Di Gioia, F., Ozores-Hampton, M., Hong, J., Kokalis-Burelle, N., Albano, J., Zhao, X., Black, Z., Gao, Z. F., Wilson, C., Thomas, J., Moore, K., Swisher, M., Guo, H. C., and Rosskopf, E. N. (2016). The Effects

of Anaerobic Soil Disinfestation on Weed and Nematode Control, Fruit Yield, and Quality of Florida Fresh-market Tomato. *Hortscience* **51**, 703-711.
Dowsett, C. A., and James, T. K. (2017). Is heat treatment a viable option for destroying weed seeds in biosecurity risk goods? *New Zealand Plant Protection* **70**.
Elema, A. G., and Scheepens, P. C. (1992). Verspreiding van onkruiden en planteziekten met dierlijke mest: een risico-analyse. pp. 76. Publicatienr. 62, PAGV, Lelystad.
Eriksson (2009). Towards the Domestication of Lepidium campestre as an undersown oilseed crop, Swedish University of Agricultural Sciences, Alnarp.
Gioia, F. D., Ozores-Hampton, M., Hong, J., Kokalis-Burelle, N., Albano, J., Zhao, X., Black, Z., Gao, Z., Wilson, C., Thomas, J., Moore, K., Swisher, M., Guo, H., and Rosskopf, E. N. (2016). The Effects of Anaerobic Soil Disinfestation on Weed and Nematode Control, Fruit Yield, and Quality of Florida Fresh-market Tomato. **51**, 703.
Grantina-Ievina, L., Ievina, B., Evelone, V., Berga, S., Kovalcuka, L., Bergspica, I., Jakovele, A., Malisevs, A., Valcina, O., Rodze, I., and Rostoks, N. (2019). Potential risk evaluation for unintended entry of genetically modified plant Propagating material in Europe through import of seeds and animal feed – the experience of Latvia. *GM Crops & Food* **10**, 159-169.
Hahn, J., Parzych, D., Schulz, J., Westerman, P. R., and Gerowitt, B. (2018). Wildflower seeds in the biogas reactor: Screening the risk of survival of different species. *In* "28th German Conference on Weed Biology and Weed Control" (H. Nordmeyer and L. Ulber, eds.), Vol. 458, pp. 41-46.
Hance, R. J., and Holly, K. (1990). "Weed Control Handbook: Principles. Eighth Edition.," Blackwell Scientific Publications, Brookline Village, MA.
Hansson, D., and Ascard, J. (2002). Influence of developmental stage and time of assessment on hot water weed control. *Weed Research* **42**, 307-316.
Hansson, D., and Mattsson, J. E. (2003). Effect of air temperature, rain and drought on hot water weed control. *Weed Research* **43**, 245-251.
Hjort-Gregersen, K. (2015). "Market overview micro scale digesters." AgroTech A/S, Denemarken.
ISF (2014). Guidelines for the Disposal of Seeds Treated with Plant Protection Products pp. 8.
Johansen, A., Nielsen, H. B., Hansen, C. M., Andreasen, C., Carlsgart, J., Hauggard-Nielsen, H., and Roepstorff, A. (2013). Survival of weed seeds and animal parasites as affected by anaerobic digestion at meso- and thermophilic conditions. *Waste Management* **33**, 807-812.
Kaovich, J., Becker, R., and Doll, J. (2005). Weed seed survival in livestock systems. (U. o. Minnesota and U. o. Wisconsin, eds.).
Kolberg, R. L., and Wiles, L. J. (2002). Effect of steam application on cropland weeds. *Weed Technology* **16**, 43-49.
Lamers, J. G., Runia, W. T., Molendijk, L. P. G., and Bleeker, P. O. (2010). Perspectives of Anaerobic Soil Disinfestation. *Vii International Symposium on Chemical and Non-Chemical Soil and Substrate Disinfestation* **883**, 277-283.
Linke, K. H. (1994). Effects of Soil Solarization on Arable Weeds under Mediterranean Conditions - Control, Lack of Response or Stimulation. *Crop Protection* **13**, 115-120.
Ludeking, D., Termorshuizen, A. J., Wubben, J., van der Wurff, A., Streminska, M., and van der Helm, F. (2013). Biologische grondontsmetting met Herbie ('Bodemresetten') als alternatief voor stomen. Rapport GTB-1272. *In* "Rapport GTB-1272", pp. 120. Productschap Tuinbouw.
Meijer, B., and Lamers, J. G. (2004). Biologische grondontsmetting. Bestrijding van bodemziekten voor een gezonde bodem. PPO nr. 415. pp. 24. Praktijkonderzoek Plant & Omgeving B.V. Bomen en Akkerbouw, Groene Ruimte en Vollegrondsgroente.
Melander, B., and Jørgensen, M. H. (2005). Soil steaming to reduce intrarow weed seedling emergence. *Weed Research* **45**, 202-211.
Melander, B., and Kristensen, J. K. (2011). Soil steaming effects on weed seedling emergence under the influence of soil type, soil moisture, soil structure and heat duration. *Annals of Applied Biology* **158**, 194-203.
Monaco, T. J., Weller, S. C., and Ashton, F. M. (2002). "Wees Science. Principles and Practices," John Wiley & Sons, Inc., New York.
Muramoto, J., Shennan, C., Fitzgerald, A., Koike, S., Bolda, M., Daugovish, O., Rosskopf, E., Kokalis-Burelle, N., and Butler, D. (2008). Effect of anaerobic soil disinfestation on weed seed germination. *In* "Proceedings of the Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions", pp. 109.1-109.3.
Naylor, R. E. L. (2008). "Weed Management Handbook, 9th Edition," Blackwell Science Ltd, Oxford.
Nishimura, A., Asai, M., Shibuya, T., Kurokawa, S., and Nakamura, H. (2015). A steaming method for killing weed seeds produced in the current year under untilled conditions. *Crop Protection* **71**, 125-131.
OGTR (2019). The Biology of Carthamus tinctorius L. (safflower). pp. 58.

Pekrun, C., Lutman, P. J. W., and Baeumer, K. (1997). Induction of secondary dormancy in rape seeds (Brassica napus L.) by prolonged imbibition under conditions of water stress or oxygen deficiency in darkness. *European Journal of Agronomy* **6**, 245-255.

Riemens, M. M., Bleeker, P. O., Groeneveld, R. M. W., and van der Weide, R. Y. (2011). Bestrijding van Knopkruid: rapportage veldexperimenten 2009 en 2010. pp. 38. Plant Research International, Agrosysteemkunde, Wageningen UR.

Riemens, M. M., Van der Weide, R., and Runia, W. T. (2008). Biology and Control of Cyperus rotundus and Cyperus esculentus, review of a literature survey. PPO report 3250100200, PRI report 3310307708. Plant Research International B.V., Wageningen.

Runia, W. T., Molendijk, L. P. G., Ludeking, D. J. W., and Schomaker, C. H. (2012). Improvement of anaerobic soil disinfestation. *Comm. Appl. Biol. Sci, Ghent University* **77**, 753-762.

Runia, W. T., Thoden, T. C., van der Wurff, A. W. G., Termorshuizen, A., Feil, H., Meints, H., and Streminska, M. A. (2014). Unravelling the mechanism of pathogen inactivation during anaerobic soil disinfestation. *In* "Proceedings of the VIIIth International Symposium on chemical and non-chemical soil and substrate disinfestation" (M. Gullino, ed.), Vol. 1044, pp. 177-193. ISHS.

Schulze, J., Brodmann, P., Oehen, B., and Bagutti, C. (2015). Low level impurities in imported wheat are a likely source of feral transgenic oilseed rape (Brassica napus L.) in Switzerland. *Environmental Science and Pollution Research* **22**, 16936-16942.

Shrestha, U., Augé, R. M., and Butler, D. M. (2016). A Meta-Analysis of the Impact of Anaerobic Soil Disinfestation on Pest Suppression and Yield of Horticultural Crops. *Frontiers in Plant Science* **7**.

Singh, A., Billingsley, K., and Ward, O. (2006). Composting: A Potentially Safe Process for Disposal of Genetically Modified Organisms. *Critical Reviews in Biotechnology* **26**, 1-16.

Spagnolo, R., Oldoni, A., Custódio, T., Reis, Â., and Machado, A. (2019). Conceptual design of a thermal weed control machine. *Ciência Rural* **49**.

Stapleton, J. J., Elmore, C. L., and DeVay, J. E. (2000). Solarization and biofumigation help disinfest soil. *California Agriculture* **54**, 42-45.

Stein, A. J., and Rodriguez-Cerezo, E. (2009). "The Global Pipeline of New GM Crops: Implications of Asynchronous Approvals for International Trade, JRC Scientific and Technical Reports." European Commission, Joint Research Centre.

Teasdale, J. R., and Mohler, C. L. (2000). The Quantitative Relationship between Weed Emergence and the Physical Properties of Mulches. *Weed Science* **48**, 385-392.

Thomashow, M. F. (1999). Plant Cold Acclimation: Freezing Tolerance Genes and Regulatory Mechanisms. *Annual Review of Plant Physiology and Plant Molecular Biology* **50**, 571-599.

van den Brand, W. G. M. (1986). Opkomstperiodiciteit bij veertig eenjarige akkeronkruidsoorten en enkele daarmee samenhangende onkruidbestrijdingsmaatregelen. Verslag nr. 53. pp. 79. Proefstation voor de akkerbouw en de groenteteelt in de vollegrond, Lelystad.

van der Schans, D., Bleeker, P., Molendijk, L., Plentinger, M., van der Weide, R., Lotz, B., Bauermeister, R., Total, R., and Baumann, D. T. (2006). "Practical weed control in arable farming and outdoor vegetable cultivation without chemicals," Wageningen UR, Applied Plant Research, Lelystad.

van Loenen, M. C. A., Turbett, Y., Mullins, C. E., Feilden, N. E. H., Wilson, M. J., Leifert, C., and Seel, W. E. (2003). Low Temperature–Short Duration Steaming of Soil Kills Soil-Borne Pathogens, Nematode Pests and Weeds. *European Journal of Plant Pathology* **109**, 993-1002.

VROM (2007). Beschikking DGM/SAS IM 06-005. pp. 29. Directorate-Generaal Milieu. Directie Stoffen, Afvalstoffen, Straling. Afdeling Straling, Nucleaire en Bioveiligheid, Den Haag.