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Differences in growth and herbicide sensitivity among *Cyperus esculentus* clones found in Belgian maize fields

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Summary

Cyperus esculentus is an invasive troublesome neophyte in many arable crops in Belgium. Applied weed control varies from field to field. One of the possible reasons for this variability might be a differential vegetative and reproductive behaviour among Belgian *C. esculentus* clones. In this study, growth characteristics and herbicide sensitivity of *C. esculentus* clones collected in Belgian maize (*Zea mays*) fields were evaluated. In a morphology Experiment, 25 clones were screened for growth characteristics and ability to set viable seeds under outdoor conditions. Dose–response experiments were conducted in the glasshouse to evaluate the effectiveness of two foliar-applied herbicides (bentazon and glyphosate) and two pre-sowing soil-incorporated herbicides (S-metolachlor and dimethenamid-P) for controlling 14 *C. esculentus* clones. Response variables were aboveground dry biomass, tuber number, tuber dry biomass and individual tuber dry weight. Clones exhibited large differences in shoot number (up to 3.1-fold), tuber dry biomass (up to 4.7-fold), tuber number (up to 3.4-fold), individual tuber dry weight (up to 4.8-fold), inflorescence number and capacity to set viable seeds. Large interclonal differences in herbicide sensitivity (up to 8.3- and 4.0-fold for aboveground dry biomass and tuber dry biomass, respectively) were observed. Contrary to foliar-applied herbicides, soil-incorporated herbicides were very effective and provided season-long *C. esculentus* control at doses below the recommended maximum field dose. However, low doses stimulated tuber formation. Future *C. esculentus* management strategies should take into account differential growth characteristics and herbicide sensitivity of *C. esculentus* clones.

Keywords: yellow nutsedge, tuber number, vegetative growth, reproduction, tuber biomass, variability.

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Introduction

Cyperus esculentus L. (yellow nutsedge) is a member of the *Cyperaceae* family that originated from (sub)tropical areas and is listed as the sixteenth worst weed in the world (Holm *et al.*, 1977). It can spread very fast, due to its high reproduction rate. One plant can produce up to 7000 tubers in one season (Stoller & Sweet, 1987). *Cyperus esculentus* is hard to control in Belgium because of its fast proliferation and few authorised herbicides. The species is an obligate outcrossing species. It can produce viable seeds, but seed production

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is very variable, and there is no evidence that seedlings can establish in cultivated areas (Mulligan & Junkins, 1976).

Cyperus esculentus was introduced into France and the Netherlands in the second half of the 20th century as a contaminant of gladiolus bulbs imported from the USA and is on the European and Mediterranean Plant Protection Organization (EPPO) list of invasive alien species (EPPO, 2016). In 1982, *C. esculentus* infestation was reported for the first time in Limburg, the easternmost province of Flanders (northern part of Belgium) (Verloove, 2010). This neophyte has been able to move from east to west, even though stringent control measures were in place over the past decades to stop its spread. As of 2016, it is locally found along roadsides and in nature reserves, where it completely outcompetes native species and thus lowering biodiversity and disrupting ecosystem functioning (EPPO, 2016). The cumulative agricultural area infested by *C. esculentus* is estimated at 16 000 ha, and the number of infested fields is still increasing (B De Cauwer pers. obs.). The rapid spread should be stopped, to prevent further economic damage to root, tuber and bulb crops. Indirectly, the spread is a menace for the potato and vegetable processing industry and international trade (through bans on contaminated produce) (EPPO, 2016). Infestation can lead to total crop loss and can interfere with crop rotation systems. The actual economic damage is dependent on cropping system, crop type, land value and phytosanitary measures. *Cyperus esculentus* infestations are estimated to have caused up to 900 € ha^{-1} y^{-1} in economic losses for a standard farm in the Netherlands (EPPO, 2016).

Since September 2015, control of *C. esculentus* has primarily focused on prevention (in particular, avoiding displacement of tubers by vehicles, machines, root crops and shoes), monitoring (early detection and within-field mapping) and intervention (eliminating tuber production). Intervention is largely based on chemical tools, mainly maize herbicides, as effectiveness of non-chemical tools is poor (Naber & Rotteveel, 1986). However, efficacy of chemical control varies greatly from field to field. This may possibly reflect morphological variation among local clones. Mulligan and Junkins (1976) reported that there are morphological and genetic differences within the North American clones of *C. esculentus* and suggested that these differences could be relevant for *C. esculentus* control. Evaluation of plant morphological characteristics by Schippers *et al.* (1995) led to the identification of differential characters, unaffected by variable growing conditions, enabling variety identification. Five taxonomic varieties are currently recognised: the wild types var. *esculentus*, var. *heermannii* (Buckley) Britton, var. *leptostachyus* Boeckeler and var. *macrostachyus* Boeckeler, and the cultivated type var. *sativus* Boeckeler. Recent field and glasshouse studies with different herbicides such as glyphosate (Felix *et al.*, 2012), glyphosate plus acetolactate synthase (ALS)-inhibiting herbicides (Nelson & Renner, 2002), mesotrione (McCurdy *et al.*, 2009) and combinations such as mesotrione with atrazine and bentazon (Armel *et al.*, 2008) have led to insights into herbicidal response regarding regrowth and tuber production. However, systematic comparison of herbicide sensitivity profiles between different biotypes has not been made, even after Pereira *et al.* (1987) reported inconsistent chemical control with atrazine due to differences in biotypes and tuber size.

In this study, two hypotheses were tested: (H1) Belgian *C. esculentus* clones exhibit large differences in vegetative and reproductive growth, and (H2) Belgian *C. esculentus* clones vary substantially in herbicide sensitivity. If large differences do exist, future weed management programmes should be tailored to the clones present in the field.

Materials and methods

Test clones

Experiments were conducted in the summer and autumn of 2015 with Belgian *C. esculentus* clones. One single mother tuber was collected in each of 25 maize fields in the spring of 2014. Fields were distributed over the five Flemish provinces (the northern part of Belgium) and were located at least 12 km apart, except for two fields. This distance was chosen to avoid sampling of fields with a common farmer or agricultural contractor. Geoposition and prevailing pedohydrological characteristics of sampling locations are provided in Table 1. Mother tubers were propagated simultaneously in 10 L pots (filled with a 1:1 mixture of sandy loam and peat) under natural *in situ* weather conditions in 2014. The resulting propagated tubers form the 25 clones, named after the village where the mother tuber was collected (Table 1). During winter, pots with tubers were kept frost free in a refrigerator set at 4–8°C until tubers were used in the experiments. At the start of the experiments, tubers were harvested and cleaned. Only medium-sized tubers were used in the experiments. Medium-sized tubers were defined as tubers with a fresh weight falling between 80% and 120% of the clone-specific mean fresh tuber weight provided in Table 1. These tubers were representative for the clone-specific tuber size. Indeed, based on the data set of 25 clones, mean fresh weight of mother tubers was positively correlated with mean individual tuber dry weight of newly produced tubers (Pearson

Table 1 Tested clones of *Cyperus esculentus* with their mean individual tuber fresh weight and corresponding sampling locations with their geographic co-ordinates, prevailing soil texture and drainage class as derived from the digital soil map of Belgium (DOV, 2017). The clones are named after the village of origin

Clone	Latitude	Longitude	Soil texture	Drainage class*	Exp. 1†	Exp. 2†	Mean individual tuber fresh weight (mg)
Aalter	51°04′55.9″N	3°24′25.1″E	Loamy sand	d	x	x	202
Ardooie	50°58′25.21″N	3°13′53.39″E	Sandy loam	d	x	x	245
Breebeek	51°09′26.8″N	5°36′29.5″E	Loamy sand	d	x		170
Desselgem	50°51′56.94″N	3°23′22.99″E	Loamy sand	d	x	x	283
Dessel	51°14′38.3″N	5°07′48.4″E	Sand	d	x		175
Evergem-Kluizen	51°09′42.3″N	3°42′49.5″E	Loamy sand	d	x	x	165
Geel	51°10′45.5″N	4°55′30.8″E	Sand	c	x	x	172
Grobbendonk	51°09′19.0″N	4°44′4.7″E	Loamy sand	c	x	x	281
Ham	51°06′8.7″N	5°10′17.0″E	Sand	c	x		159
Houthalen	51°02′3.3″N	5°23′47.7″E	Sand	c	x	x	140
Koekelare	51°04′34.50″N	2°59′46.87″E	Loamy sand	d	x		243
Lommel	51°15′21.2″N	5°24′10.2″E	Sand	c	x	x	190
Maaseik	51°06′57.6″N	5°43′37.4″E	Loamy sand	d	x	x	159
Maria-Aalter	51°04′54.6″N	3°26′26.9″E	Sand	d	x	x	186
Meulebeke	50°58′09.70″N	3°19′13.44″E	Sandy loam	d	x		474
Oostkamp	51°06′46.92″N	3°14′41.38″E	Sand	c	x	x	378
Overmere	51°02′08.8″N	3°56′38.6″E	Sand	c	x		220
Poppel	51°26′24.2″N	5°01′18.7″E	Sand	d	x	x	180
Sinaai-Waas	51°09′06.1″N	4°00′10.2″E	Sand	b	x		148
Sint-Niklaas	51°12′7.9″N	4°11′4.4″E	Loamy sand	b	x		255
Snellegem	51°09′49.93″N	3°07′41.31″E	Sand	d	x		257
Ternat	50°51′22.6″N	4°10′59.5″E	Silt loam	a	x	x	132
Waregem1	50°54′08.96″N	3°26′59.83″E	Loamy sand	d	x		223
Waregem2	50°52′14.5″N	3°22′51.7″E	Sandy loam	d	x		350
Wielsbeke	50°55′40.48″N	3°21′42.25″E	Sandy loam	c	x	x	99

*Drainage classes: a, excessively drained; b, well drained; c, moderately well drained; d, imperfectly drained.
†x, included in the experiment.

product–moment correlation coefficient of 0.97 and P value <0.001) but was unrelated (P-values >0.05) to all other growth characteristics determined in the outdoor morphology experiment.

During the propagation phase, all collected clones were classified down to variety level, according to the identification key of Schippers *et al.* (1995), which is mainly based on individual flower characters (e.g. dimensions of floral scales, style length). Twenty-two of twenty-five clones used in this study belonged to *C. esculentus* var. *leptostachyus*. This variety was also reported as the most common variety in the Netherlands, France and the USA (Schippers *et al.*, 1995; Dodet *et al.*, 2008). Three clones (Houthalen, Maaseik and Breebeek) could not be identified to the variety level, as they did not develop inflorescences under Belgian outdoor conditions.

Outdoor morphology experiment (Experiment 1)

To support hypothesis 1, the clones were screened for their growth characteristics and ability to set viable seeds under natural outdoor conditions. Mean daily temperature, humidity and solar radiation during the experimental period are given in Table S1. The experimental design was a completely randomised block with 25 clones in six replicates. The experimental unit was a 5.3 L pot, filled with steamed sandy loam soil containing 2.6% organic matter, 46.7% silt (2–50 μm), 43.4% sand (>50 μm) and 10.0% clay with a pH-KCl of 5.5, and planted with one pre-sprouted tuber at 4 cm depth, on the 15 April. Tubers were pre-sprouted (BBCH07, i.e. beginning of sprouting) in Petri dishes lined with moistened 9 cm-diameter Rotilabo filter paper (type 112A) in a germination chamber under a 15/25°C night/day temperature regime. Pots were irrigated by overhead sprinklers as needed and were not fertilised.

On the 29 September, 167 days after planting, the following growth characteristics were determined for each pot: shoot number, aboveground dry biomass, tuber dry biomass, tuber number, tuber dry weight and inflorescence number. Shoots and inflorescences were clipped 10 mm above soil surface level and

counted. Clipped biomass was dried for 16 h at 75°C to determine aboveground dry biomass. Next, all newly formed tubers (mature as well as immature) were washed out of the pot substrate by use of a 200 μm sieve and counted. Tubers were dried for 16 h at 75°C to determine tuber dry biomass per pot. Individual tuber dry weight was calculated as the ratio of tuber dry biomass to tuber number.

To assess the ability to set viable seeds, seeds were collected from clipped inflorescences (29 September), bulked per clone, stored for 2 months in a refrigerator set at 2°C and subjected to a germination test (1 December). Per clone, four seedlots of 100 seeds each were exposed to 16 h light/8 h darkness and a 15/25°C day/night temperature regime for 28 days.

Glasshouse dose–response pot experiments (Experiment 2)

To evaluate hypothesis 2, dose–response pot experiments were conducted to assess variability in herbicide sensitivity among clones. Intraspecific variability was tested by subjecting 14 clones (approx. two to three clones per province) to two foliar-applied herbicides [bentazon (Basagran, 87%, SG, Basf Belgium) and glyphosate (Roundup Powermax, 480 g L-1, SL, Monsanto Europe)] (hereafter named Exp. 2.1) and two preplant-incorporated soil-acting herbicides [S-metolachlor (Dual Gold, 960 g L-1, EC, Syngenta Crop Protection) and dimethenamid-P (Frontier Elite, 720 g L-1, EC, Basf Belgium)] (hereafter named Exp. 2.2). The selected herbicides are all single active ingredients that are most effective in controlling *C. esculentus* and authorised for use in Belgium. Bentazon, S-metolachlor and dimethenamid-P are frequently and widely applied in Belgian maize fields. Glyphosate is frequently used as part of a chemical fallow programme in fields infested with *C. esculentus* or for spot application.

Both subexperiments were conducted in glasshouses using plastic pots filled with steamed sandy loam soil (the same as in Experiment 1). The glasshouse was a rain-shelter plastic glasshouse, with sides left open up to 1 m high for natural ventilation. Mean daily temperature, humidity and solar radiation during the experimental periods are given in Table 2. Pots were irrigated by overhead sprinklers as needed. The experimental design of each subexperiment was a randomised block with three replicates. The experimental unit was one 640 ml plastic pot (Ø = 9 cm, ht = 10 cm) with three pre-sprouted (as described in experiment 1) mother tubers planted at 4 cm depth. Each herbicide was tested in seven or eight doses and compared to a control as enumerated in Table 2. Dose ranges listed in Table 2 allowed successful fitting of dose–response curves in preliminary experiments (data not shown). In Experiment 2.1, foliar-applied herbicides were applied at the five-leafs stage (BBCH 15) of *C. esculentus*, that is the weed growth stage at which post-emergence herbicides with activity against *C. esculentus* are most commonly applied in Belgian maize fields. Herbicides were applied with TeeJet XR11002 flat fan nozzles (TeeJet Technologies, Wheaton, IL, USA) at a spray pressure of 180 kPa and a spray volume of 300 L ha^{-1}. In Experiment 2.2, herbicides were uniformly mixed into the soil prior to filling of the pots, to assure herbicide presence in deeper soil layers. Prior to incorporation, the steamed soil was sieved through a 2 mm mesh screen and air-dried. Next, the air-dry soil (in g) was mixed with a dose-dependent aqueous herbicide solution (in ml) at a ratio of 1:0.1 and transferred into the pots (890 g soil pot^{-1} with a soil density of 1.4 g cm^{-3}). Herbicides were applied at doses ranging from 0.024 to 0.771 mg dimethenamid-P kg^{-1} air-dry soil and 0.023–1.097 mg S-metolachlor kg^{-1} air-dry soil, corresponding to doses of 33.75–1080 g dimethenamid-P ha^{-1} and 48–1536 g S-metolachlor ha^{-1} incorporated into a depth of

Table 2 Herbicides and their doses examined in post-emergence (POST) and preplant-incorporated (PPI) dose–response bioassays in 2015 (Experiment 2)

Experiment	Herbicide*	Application	Maximum field dose (g a.i. ha^{-1})	Herbicide dose (g a.i. ha^{-1})
2.1	Bentazon†	POST	696	0, 100, 200, 400, 800, 1600, 3200, 6400
	Glyphosate	POST	1440	0, 180, 360, 720, 1440, 2880, 5760
2.2	Dimethenamid-P	PPI (0–10 cm)	1000	0, 33.75, 67.5, 135, 270, 540, 1080
	S-metolachlor	PPI (0–10 cm)	1536	0, 48, 96, 192, 384, 768, 1536

*Formulated products: bentazon (Basagran, 87%, SG, Basf Belgium), glyphosate (Roundup Powermax, 480 g L^{-1}, SL, Monsanto Europe), dimethenamid-P (Frontier Elite, 720 g L^{-1}, EC, Basf Belgium), S-metolachlor (Dual Gold, 960 g L^{-1}, EC, Syngenta Crop Protection).

†1 L ha^{-1} of triglyceride oil (Actirob B, 812 g a.i. L^{-1}, EC, Novance), a methylated seed oil, was added to the herbicide spray solution to enhance foliar uptake and distribution.

10 cm. Experiments were not repeated, in time as they were only designed to assess the existence of interclonal variation in herbicide sensitivity.

Plant responses to herbicides were assessed through determination of aboveground dry biomass (Exp. 2.1 and Exp. 2.2), tuber dry biomass (Exp. 2.2), tuber number (Exp. 2.2) and individual tuber dry weight (Exp. 2.2) per pot. Response variables were determined 28 (Exp. 2.1) and 134 (Exp. 2.2) days after herbicide application. To determine aboveground dry biomass per pot, plants were clipped 5 mm above soil level and oven-dried for 16 h at 75°C. All formed tubers were harvested per pot by washing out the pot substrate through a sieve with a mesh width of 200 μm. After washing, tubers were counted and dried for 16 h at 75°C to determine tuber dry biomass per pot and tuber dry weight.

Data analysis

All data were analysed in R version 3.2.0. (R Core Team, 2015). The normality and homoscedasticity were checked with a Q-Q plot and a Levene test respectively. No data transformation was required.

In Experiment 1, all data (aboveground dry biomass, shoot number, tuber dry biomass, tuber number, individual tuber dry weight, number of inflorescences and seed germination) were analysed using one-way ANCOVA with mean fresh weight of the mother tuber as covariable. All germination data were expressed as percentages. Differences between treatment means were compared using Tukey's HSD test at the 5% significance level. To study the strength of the relationships between measured morphological variables, the Pearson product–moment correlation coefficient was determined.

In Experiment 2, data (aboveground dry biomass, tuber dry biomass, tuber number, individual tuber dry weight) obtained from dose–response bioassays were analysed with the drc package (Ritz & Streibig, 2005). Dose–response curves were calculated according to Streibig *et al.* (1993). Within Experiment 2, dose–response curves for all clones were fitted simultaneously for each tested herbicide. Effective dosage ED_{90} (dose required for 90% reduction in aboveground biomass, etc.) and selectivity indices (SI) as relative potencies between two dose–response curves were derived from the regression model utilising the delta method (van der Vaart, 1998). The SI (90, 90), that is the ratio between ED_{90} for one dose–response curve and ED_{90} for another dose–response curve, was used to compare the relative differences of ED_{90} doses among curves. Models used for dose–response curve fitting are provided in Tables S2–S6. To examine the relationship between ED_{90} values obtained for each herbicide and measured morphological variables, Pearson product–moment correlation coefficients were determined. To assess the effect of geographic distance between clone origins on morphology and herbicide response, simple Mantel tests (Mantel, 1967) were performed. Permutation Mantel tests with 9999 random permutations were run separately for each morphological variable and ED_{90} response variable.

Results

Morphological variability (Exp. 1)

Aboveground dry biomass (Figure S1A), shoot number (Figure S1B), tuber dry biomass (Figure S1C), tuber number (Fig. 1A) and individual tuber dry weight (Fig. 1B) varied significantly across clones, with up to 2.4-, 3.1-, 4.7-, 3.4- and 4.8-fold differences between minimum and maximum values given in Table 3 respectively.

All clones except Breebeek, Houthalen and Maaseik produced inflorescences, albeit to a varying degree from less than 1 (Evergem-Kluizen) up to 13 (Koekelare) inflorescences per pot (Table 3). All clones that formed inflorescences produced viable seeds, except for the clones Evergem-Kluizen and Lommel. Germination of harvested achenes varied substantially among clones from 0 (Evergem-Kluizen, Lommel) to 88% (Aalter) (Fig. 2).

Tuber dry biomass was positively correlated with aboveground dry biomass, shoot number, tuber number and tuber dry weight (Table 4). Tuber number was negatively correlated with tuber dry weight. The clone with the lowest reproductive capacity, namely Meulebeke, produced the heaviest tubers with an average tuber dry weight of 251.9 mg (Fig. 1B, Table 3). There was also a strong positive correlation between shoot number and tuber number. Interclonal morphological differences were not correlated with geographic distance between clones, irrespective of morphological variable, except for aboveground dry biomass and number of inflorescences (Table 5).

Aboveground plant responses to herbicides (Experiment 2.1 and Experiment 2.2)

Aboveground dry biomass monotonically decreased with increasing herbicide dose, as illustrated for three clones in Fig. 3. Clones exhibited large differences in sensitivity to foliar-applied herbicides bentazon and glyphosate. The ED_{90} doses (based on aboveground dry biomass reduction) varied significantly across clones (Table S2), with up to 2.6- and 8.3-fold differences between minimum and maximum values

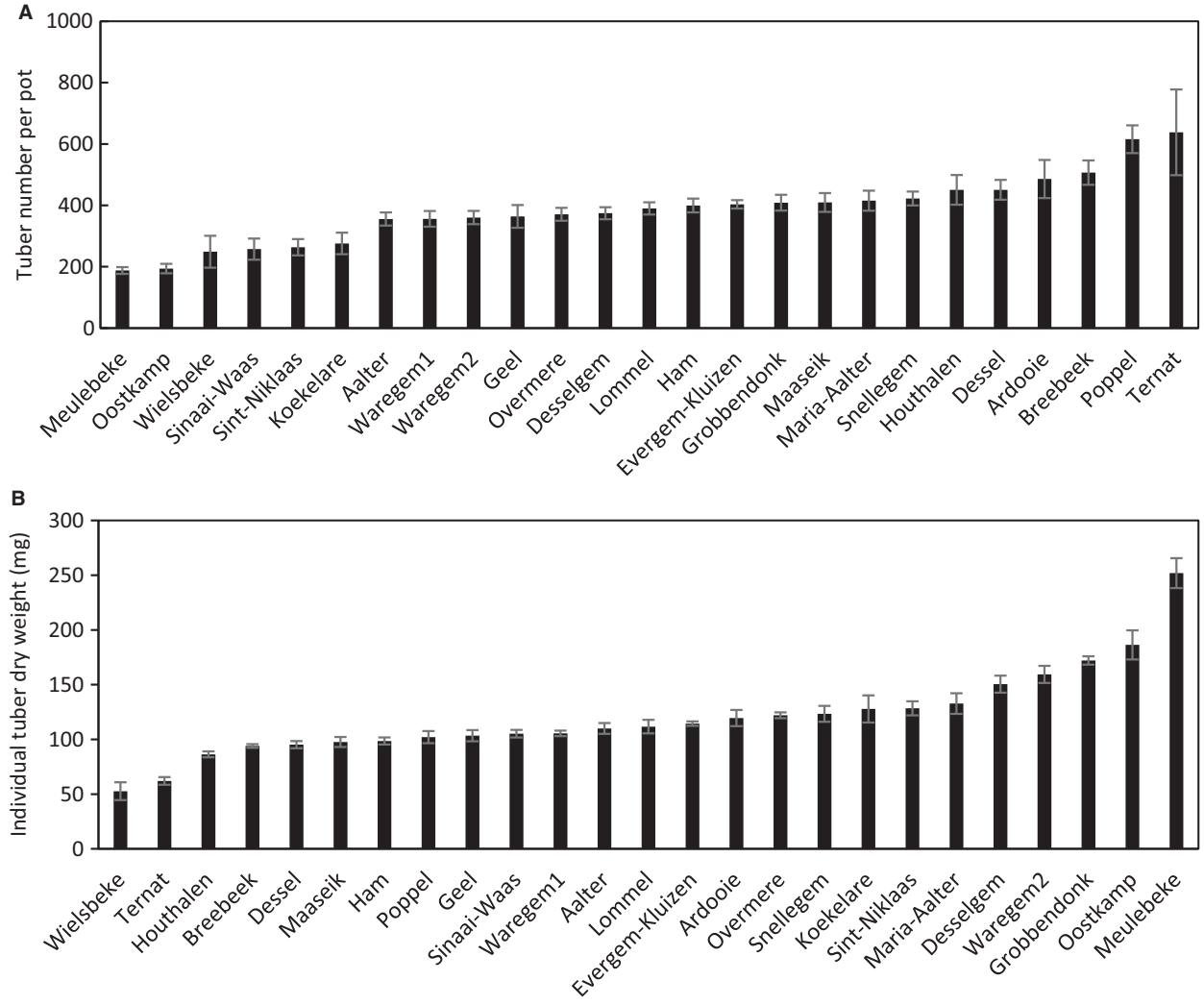

Fig. 1 Means (six replicates) and standard errors (error bars) of belowground vegetative growth characteristics of 25 Belgian *C. esculentus* clones grown outdoors in 5.3 L pots (Experiment 1). (A) Tuber number and (B) individual tuber dry weight. Clones were ranked in ascending order based on their plant response.

Table 3 Minimum, maximum and median for various growth characteristics and seed germination determined on a set of 25 Belgian *Cyperus esculentus* clones grown outdoors in 5.3 L pots (Experiment 1)

Plant feature	Minimum*	Maximum*	Median
Aboveground dry biomass (g pot^{-1})	14 (Houthalen)	33 (Geel)	25
Shoot number	29 (Wielsbeke)	91 (Poppel)	54
Tuber dry biomass (g pot^{-1})	15 (Wielsbeke)	71 (Grobbendonk)	43
Tuber number	187 (Meulebeke)	638 (Ternat)	390
Individual tuber dry weight (mg)	52.7 (Wielsbeke)	251.9 (Meulebeke)	112
Inflorescence number	0 (Breebeek, Houthalen, Maaseik)	13 (Koekelare)	5
Seed germination (%)	0 (Evergem-Kluizen, Lommel)	88 (Aalter)	20

*Clones corresponding to these minimum and maximum values are given between brackets. Min. and max. values are all significantly different at $P = 0.05$ according to the Tukey's HSD test.

(Table 6) for bentazon and glyphosate respectively. All clones had ED_{90} doses higher than the maximum field doses of bentazon and glyphosate allowed in Belgium (*i.e.* 696 and 1440 g a.i. ha^{-1}, respectively), except for clone Aalter treated with glyphosate.

Similar to foliar-applied herbicides, ED_{90} doses for preplant-incorporated (PPI) herbicides dimethenamid-P and S-metolachlor varied significantly across clones (Table S3), with up to 4.0- and 3.1-fold differences between minimum and maximum values (Table 6)

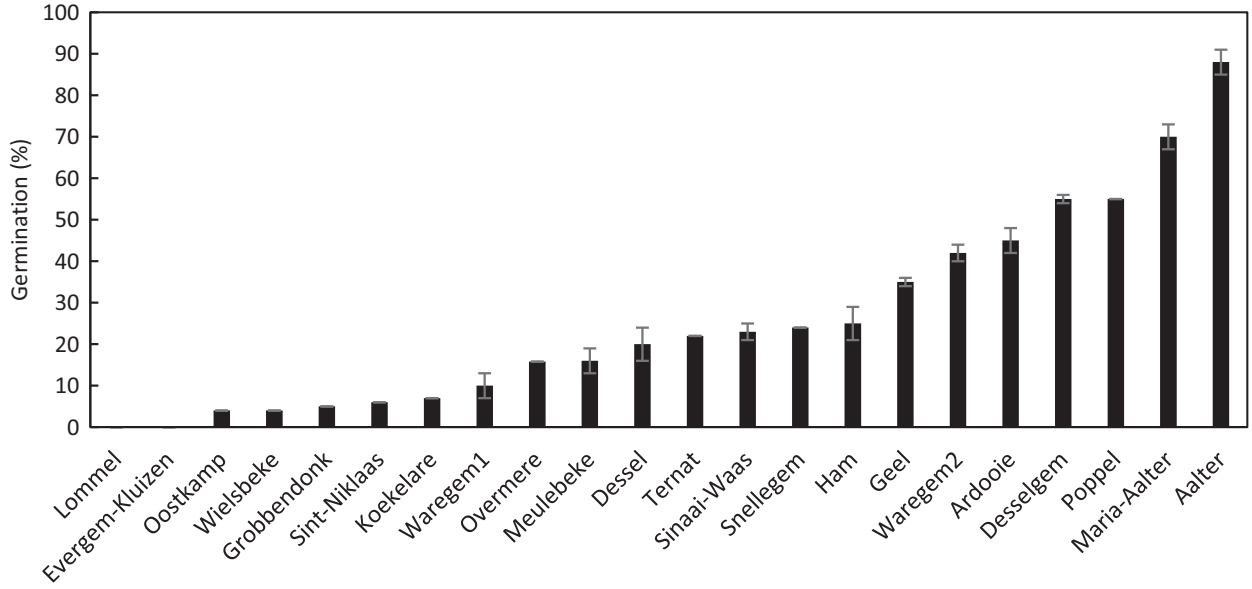

Fig. 2 Germination (%) from seeds produced by *C. esculentus* clones (Experiment 1). Data are means of 4* 100 seeds. Error bars are standard errors. Clones were ranked in ascending order.

Table 4 Pearson's correlation coefficients between various growth characteristics of a set of 25 Belgian *Cyperus esculentus* clones (Experiment 1)

	Aboveground dry biomass	Shoot number	Tuber dry biomass	Tuber number	Individual tuber dry weight	Inflorescence number
Aboveground dry biomass	1.00	0.45***	0.51***	0.24**	0.24**	0.37***
Shoot number		1.00	0.47***	0.58***	−0.13	0.09
Tuber dry biomass			1.00	0.57***	0.40***	0.08
Tuber number				1.00	−0.40***	−0.10
Individual tuber dry weight					1.00	0.18*
Inflorescence number						1.00

Significance of coefficients is indicated as ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

Table 5 Mantel test results for among-clone differences in growth characteristics, and geographic distance matrices

Growth characteristic	Geographic distance
Aboveground dry biomass	0.227**
Shoot number	0.091
Tuber dry biomass	−0.083
Tuber number	0.065
Individual tuber dry weight	0.017
Inflorescence number	0.194*

Values represent Mantel correlation coefficients (r) based on Pearson product–moment correlation coefficients, and significance was examined using Monte Carlo permutation tests (**$P < 0.01$, *$P < 0.05$).

respectively. All clones were controlled by doses below maximum authorised field dose of dimethenamid-P and S-metolachlor (i.e. 1000 and 1536 g a.i. ha^{-1} respectively).

Belowground plant responses to herbicides (Experiment 2.2)

Similar to ED_{90} dose results based on aboveground dry biomass, ED_{90} doses calculated on the basis of tuber dry biomass, tuber number and individual tuber dry weight varied significantly across clones (Tables S4, S5 and S6). For PPI dimethenamid-P and S-metolachlor, ED_{90} doses of the most sensitive and least sensitive clone, based on tuber dry biomass, differed 3.6- and 4.0-fold respectively (Table 6 and Table S4). All clones had ED_{90} doses lower than maximum authorised field dose of dimethenamid-P and S-metolachlor in Belgium (i.e. 1000 and 1536 g ha^{-1} respectively). For PPI dimethenamid-P and S-metolachlor, differences in ED_{90} doses, based on tuber number, were up to 4.2- and 7.7-fold respectively (Table 6 and Table S5). All clones had ED_{90} doses lower than maximum field dose of dimethenamid-P and S-metolachlor

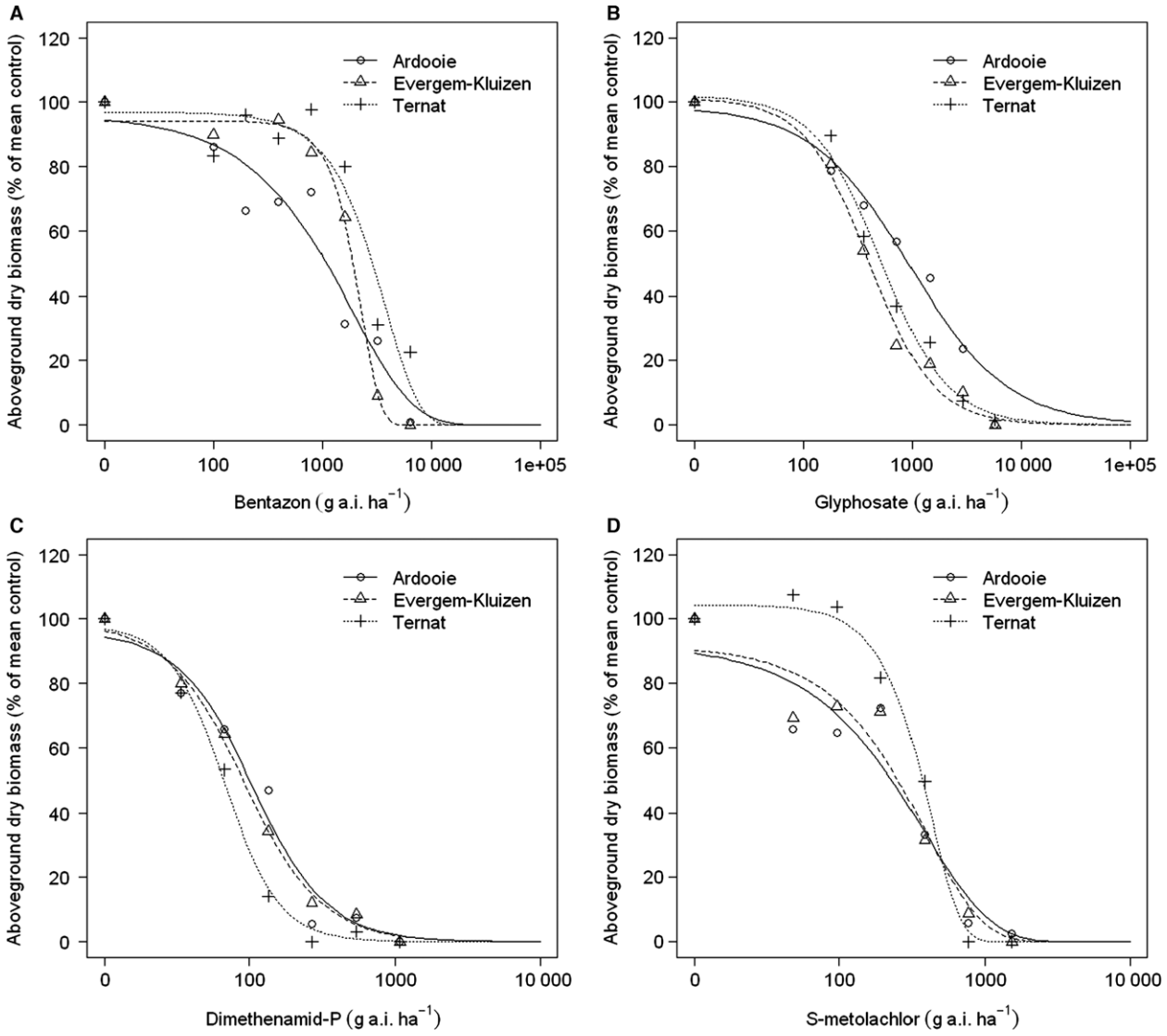

Fig. 3 Aboveground dry biomass produced by the Belgian *C. esculentus* clones Ardooie, Evergem-Kluizen and Ternat in response to increasing bentazon (A), glyphosate (B), dimethenamid-P (C) and S-metolachlor (D) doses (Experiment 2.1 and Experiment 2.2). Observed data (symbols) are % of untreated control, and lines are obtained by simultaneous fitting procedure proposed by Ritz and Streibig (2005).

Table 6 Minimum, maximum and median for ED_{90} responses to the foliar-applied herbicides bentazon and glyphosate applied at the five-true-leaf growth stage (Experiment 2.1) and the preplant-incorporated herbicides dimethenamid-P and S-metolachlor (Experiment 2.2) determined on a set of 14 Belgian *Cyperus esculentus* clones

Herbicide	Response variable	Minimum*	Maximum*	Median
Bentazon	Aboveground dry biomass	3239 (Evergem-Kluizen)	8462 (Oostkamp)	5507
Glyphosate	Aboveground dry biomass	1111 (Aalter)	9207 (Ardooie)	4587
Dimethenamid-P	Aboveground dry biomass	150 (Aalter)	601 (Houthalen)	279
	Tuber dry biomass	91 (Lommel)	331 (Houthalen)	161
	Tuber number	163 (Maaseik)	689 (Oostkamp)	234
	Individual tuber dry weight	141 (Wielsbeke)	1831 (Oostkamp)	359
S-metolachlor	Aboveground dry biomass	315 (Wielsbeke)	979 (Houthalen)	639
	Tuber dry biomass	198 (Wielsbeke)	793 (Oostkamp)	390
	Tuber number	220 (Wielsbeke)	1691 (Houthalen)	924
	Individual tuber dry weight	339 (Wielsbeke)	2506 (Oostkamp)	648

*Clones corresponding to these minimum and maximum values are given between brackets. Min. and max. values are all significantly different at $P = 0.05$ based on computed selectivity indices and corresponding P-values.

in Belgium, except for clone Houthalen treated with S-metolachlor. For PPI dimethenamid-P and S-metolachlor, differences in ED_{90} doses, based on tuber dry weight, were up to 13.0- and 7.4-fold respectively (Table 6 and Table S6). All clones had ED_{90} doses lower than maximum authorised field dose of dimethenamid-P and S-metolachlor in Belgium, except for Desselgem and Houthalen for dimethenamid-P, and Oostkamp for dimethenamid-P and S-metolachlor.

Low dosages of dimethenamid-P and S-metolachlor stimulated the number of tubers produced, as shown by the hormetic dose–response curves in Fig. 4 and positive values obtained for parameter f of the Brain–Cousens hormesis model (Table S5). Such hormetic responses are found at less than or about 135 g ha^{-1} of dimethenamid-P and 192 g ha^{-1} of S-metolachlor, depending on clone. For example, dimethenamid-P-caused hormesis is found at about 135 g a.i. ha^{-1} in clone Ardooie, but at about 33.75 g a.i. ha^{-1} in clone Evergem-Kluizen. The maximum stimulatory effect of low dosages ranged between 5 and 65% for dimethenamid-P, and between 20 and 160% for S-metolachlor, depending on clone. No hormetic responses were found for tuber dry biomass and individual tuber dry weight, for which monotonically decreasing log-logistic dose–response functions were obtained as illustrated for three clones in Fig. 4.

The ED_{90} responses to foliar-applied herbicides bentazon and glyphosate were not intercorrelated, nor were they individually related to the ED_{90} responses to dimethenamid-P and S-metolachlor (Table S7). In general, the ED_{90} responses for aboveground dry biomass, tuber dry biomass, tuber number and individual tuber dry biomass were highly positively intercorrelated within and between preplant-incorporated herbicides dimethenamid-P and S-metolachlor.

Morphological data and ED_{90} response data were not related, irrespective of morphological variable and herbicide, except for individual tuber dry weight, which was positively correlated with ED_{90} response to dimethenamid-P based on individual tuber dry weight and tuber number. Dissimilarity in herbicide response among clones was not correlated with geographic distance between clones, irrespective of herbicide (Table S8).

Discussion

Hypothesis 1 is supported. *Cyperus esculentus* clones found in Belgian maize fields revealed significant variation in vegetative growth and reproductive capacity. Differences in aboveground dry biomass, shoot number, tuber dry biomass, tuber number and tuber dry weight up to 2.4-, 3.1-, 4.7-, 3.4- and 4.8-fold, respectively, were observed among clones. Furthermore, clones significantly differed in their capacity to form inflorescences and viable seeds. Aforementioned large interclonal variability points to a presumed differential genetic background of local clones found in Belgium. Mantel tests revealed no significant relationships between clone morphology and geographic origin, except for aboveground dry biomass and number of inflorescences. Hence, the omnipresence of *C. esculentus* clones across Belgian arable land most probably cannot solely be explained by a massive spread of a few clones introduced decades ago. Most likely, new clones with a different genetic background are continuously accidentally introduced in Flanders through diverse man-made pathways such as shipping, agriculture, horticultural trade [e.g. importation of infested Mediterranean container plants as reported by Hoste *et al.* (2008) and floral bulbs], recreation (e.g. use of *C. esculentus* tubers as fishing bait), travel and tourism, thus further increasing genetic variability among clones. This variability is not in line with Dodet *et al.* (2008), who only found small genetic variability among local *C. esculentus* clones found in a region in southwest France.

Prolific tuber production was observed among container-grown Belgian clones with a maximum vegetative reproduction capacity of 1:638 (Ternat), in line with Bohren and Wirth (2015) who found a reproduction factor of 1:746 for a particular Swiss container-grown *C. esculentus* clone. However, sexual reproduction under Belgian weather conditions cannot be excluded. Indeed, in our outdoor container experiment, 84% of the tested clones were able to produce viable seeds under prevailing Belgian weather conditions, albeit to a varying degree (between 0 and 88%) with an average germination of 29%. This was in line with Hill *et al.* (1963) who reported a germination percentage of 51% and with Thullen and Keeley (1979) who found germination percentages between 20 and 70%. This successful seed set is most probably due to the fact that all clones were grown together in close proximity, as *C. esculentus* is known as a wind-pollinated, self-incompatible species (Tayyar *et al.*, 2003). Due to the steady increase in number of infested fields and large morphological variability among local clones in Belgium, the probability of finding genetically different clones growing in close proximity has certainly increased. As a consequence, sexual reproduction in field circumstances is highly likely. So, successful seed set may be another important factor contributing to *C. esculentus* spread, provided that seeds survive harsh winter conditions. The small achenes of *C. esculentus* may be more easily transported (through soil attached to shoes or agricultural machinery) from field to field

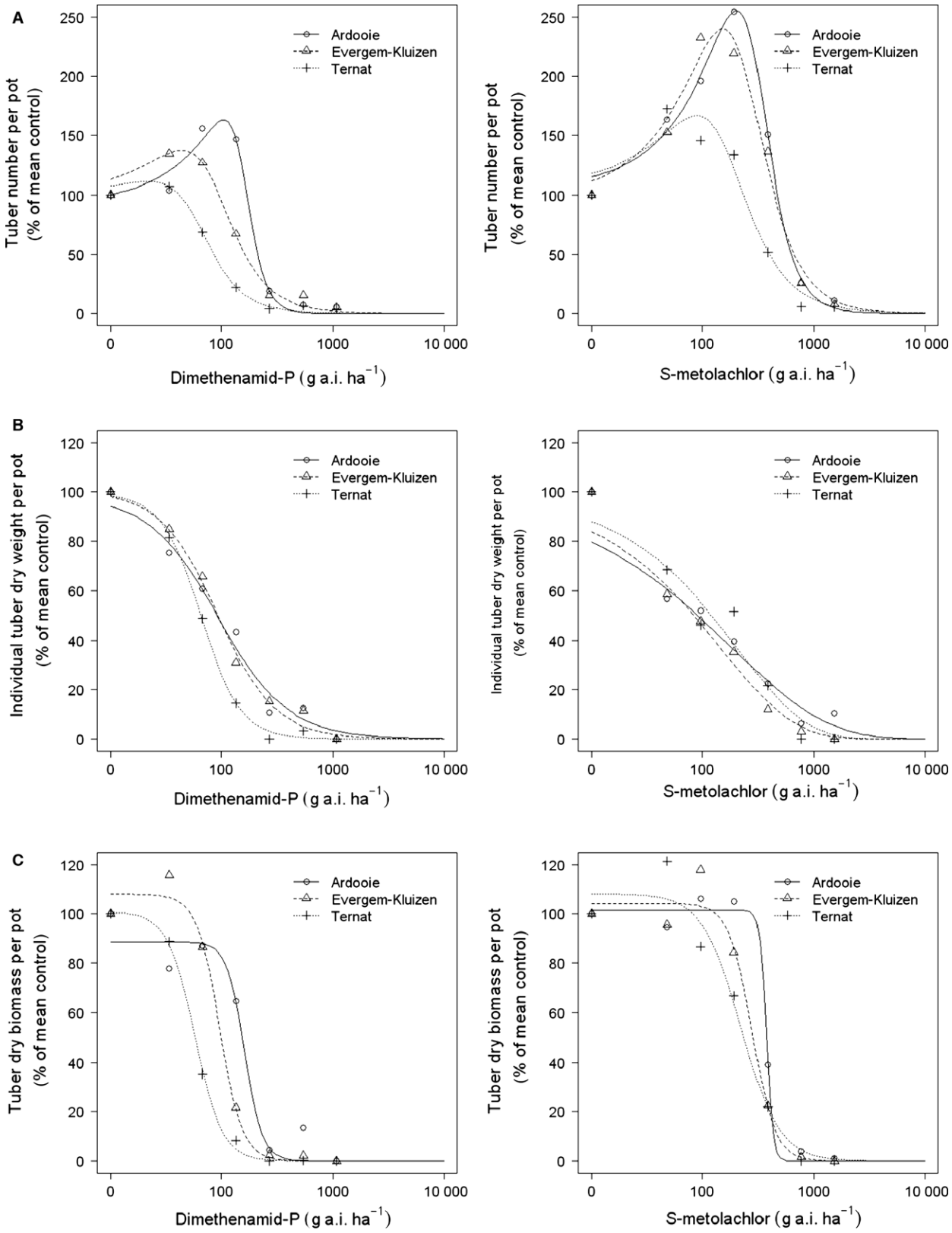

Fig. 4 Tuber number (A), individual tuber dry weight (B) and tuber dry biomass (C) produced by the Belgian *C. esculentus* clones Ardooie, Evergem-Kluizen and Ternat in response to increasing dimethenamid-P and S-metolachlor doses (Experiment 2.2). Observed data (symbols) are % of untreated control, and lines are obtained by simultaneous fitting procedure proposed by Ritz and Streibig (2005).

than tubers. Fortunately, seedlings may be more easily controlled than *C. esculentus* plants grown from mother tubers, as they are not connected with a mother tuber that fosters initial growth and may enable resprouting after chemical control of aboveground biomass (pers. obs. B De Cauwer). However, seedlings growing in non-agricultural zones (e.g. road verges, ditch banks, nature reserves) mostly do not receive herbicides and, hence, may contribute to *C. esculentus* spread and variability.

Hypothesis 2 is supported. *Cyperus esculentus* clones exhibited pronounced interclonal variability in herbicide sensitivity. Doses required for 90% reduction in aboveground dry biomass varied up to 2.6-, 8.3-, 4.0- and 3.1-fold for foliar-applied bentazon, foliar-applied glyphosate, PPI dimethenamid-P and PPI S-metolachlor respectively. Hence, interclonal variation in herbicide activity is highest for the systemic herbicide glyphosate and lowest for the contact herbicide bentazon. Both foliar herbicides exhibited poor activity against all *C. esculentus* clones tested, as doses up to 12 (bentazon) and 6.4 (glyphosate) times higher than the maximum authorised field doses (i.e. 696 g a.i. ha^{-1} for bentazon and 1440 g a.i. ha^{-1} for glyphosate) in Belgium were required for 90% control. Our results are in line with Stoller *et al.* (1975), who reported unsatisfactory *C. esculentus* control in the field with glyphosate rates below 2200 g a.i. ha^{-1}. Indeed, in their field experiment, doses of 800 and 1700 g ha^{-1} bentazon resulted in 33 and 73% control, respectively, 4 weeks after application. Poor activity of these foliar-applied herbicides may be explained by poor herbicide absorption. *Cyperus esculentus* has a thick waxy cuticle on the adaxial leaf surface (Schippers *et al.*, 1995), which may present a barrier for herbicide absorption. Low absorption rate of herbicides may be improved by addition of adjuvants to the tank mix. In a study by Felix *et al.* (2012), the addition of a non-ionic surfactant to glyphosate plus ammonium sulphate resulted in the greatest *C. esculentus* foliar injury. Etheridge and Mueller (1998) and Rotteveel and Naber (1986) found that sequential applications of glyphosate and bentazon were required to provide effective *C. esculentus* control. Good coverage of the foliage by bentazon sprays is essential, because the bentazon frequently kills only the foliage contacted by the spray (Stoller *et al.*, 1975).

In contrast to foliar-applied herbicides, both preplant-incorporated soil-acting herbicides dimethenamid-P and S-metolachlor were highly active against all clones tested, with ED_{90} doses for aboveground dry biomass 134 days after incorporation of less than 64% of their maximum field doses. Due to their high activity against *C. esculentus*, the observed variation in sensitivity is less of an issue. At the maximum field dose, 11 of 14 clones produced no aboveground biomass. In the rare cases of biomass production (e.g. clone Oostkamp), the number of produced tubers and their weight were very low (see Tables S5 and S6, Figs. 3 and 4).

The interclonal differences in herbicide sensitivity were not related to geographic distance between clone origins, as shown by Mantel tests. Moreover, *C. esculentus* clones exhibited differential herbicide sensitivity profiles as sensitivity to bentazon, glyphosate and preplant-incorporated herbicides as a group was generally not intercorrelated. As a result, control levels obtained by a similar *C. esculentus* control strategy may vary from field to field, even if located in close spatial proximity.

As long-term *C. esculentus* management is dependent on inhibiting tuber production (Felix *et al.*, 2012; Bohren & Wirth, 2015), herbicidal responses on tuber formation are of crucial importance. From the results obtained with preplant-incorporated herbicides, it can be deduced that total tuber biomass and individual tuber dry weight monotonically decreased with increasing dose rate of dimethenamid-P and S-metolachlor. This is simply the reflection of the decrease in aboveground dry biomass and concurrent decrease in photoassimilates: according to Bhowmik (1997), tuber production in *C. esculentus* is a plant response to excess carbohydrates and is regulated by the availability of growth substances. However, contrary to total tuber dry biomass and individual tuber weight, tuber number did not monotonically decrease with increasing dose rate; at low dose rates (less than or about 135 and 192 g ha^{-1} of dimethenamid-P and S-metolachlor, respectively), clone-dependent hormetic effects were observed. Plants treated with hormetic doses produced more tubers albeit smaller weight compared with untreated ones. Presumably, low dosages of dimethenamid-P and S-metolachlor stimulate tuber formation as a survival mechanism. The lower individual tuber dry weights could be explained by the herbicide-induced reduction in foliar biomass (for which no hormesis effect was noticed) and concurrent limitation in the amount of photoassimilates. As a result of the limited amounts of photoassimilates and higher tuber number, final individual tuber weights obtained 134 days after incorporation were smaller. Doses higher than the hormetic doses will further reduce aboveground foliage dry biomass, and hence, fewer and smaller tubers will be formed as levels of assimilates are too low to sustain the tuberisation process. Due to the hormetic plant responses, the use of low dimethenamid-P and S-metolachlor dosages should be discouraged to avoid increases in the number of

C. esculentus tubers in infested fields. As residual activity of dimethenamid-P and S-metolachlor will drop over time through decomposition or leaching, herbicide concentrations fostering hormetic responses will be reached earlier in the growth season after low-dose application than after high-dose application, particularly in heavy-textured soils exhibiting high capacity for herbicide adsorption. This is of particular relevance for late germinating or resprouting tubers, and for clones producing heavy mother tubers, as sensitivity to dimethenamid-P, in terms of reduction in tuber number, was positively correlated with the clone-specific weight of mother tubers (Table S7). In addition, these soil-acting herbicides should be uniformly incorporated into the topsoil; otherwise, some sprouting mother tubers will be exposed to varying dosages, including hormetic dosages. As most tubers emerge from the 0–10 cm top soil (Tumbleson & Kommedahl, 1961), incorporation depth should be about 10 cm. The reduction in both individual tuber dry weight and number of newly produced tubers at high dosages of PPI dimethenamid-P and S-metolachlor is very important for long-term *C. esculentus* control. Indeed, at high doses, few new tubers are formed and they remained small. As longevity of small-sized tubers is much lower than that of large-sized tubers (Thullen & Keeley, 1979), the bud bank of *C. esculentus* can be exhausted over the long term, provided effective and continuous control is sustained over consecutive years.

In conclusion, interclonal variation in morphology and herbicide sensitivity found in Belgian *C. esculentus* clones may affect success of future *C. esculentus* control strategies. A strong negative correlation was found between tuber number and individual tuber dry weight. Clones with many but small tubers may spread faster than clones with few but large tubers but may be easier to control due to their lower regrowth capacity. *Cyperus esculentus* plants should be controlled before flowering to avoid the chance of seed setting and further spread. Variability in herbicide sensitivity may complicate the appropriate choice of herbicides and their dosages. The most promising short-term way to manage *C. esculentus* seems yearly application of preplant-incorporated dimethenamid-P or S-metolachlor. Although both chloroacetamide herbicides carry a low risk of resistance development (Heap, 2017), their prolonged use should be discouraged.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Means (6 replicates) and standard errors (error bars) of vegetative growth characteristics of 25 Belgian *C. esculentus* clones grown outdoors in 5.3 L pots (Experiment 1). (A) above-ground dry biomass, (B) shoot number and (C) tuber dry biomass. Clones were ranked in ascending order based on their plant response.

Table S1 Mean daily temperature, relative humidity and solar radiation during the dose-response pot experiments in 2015.

Table S2 Regression model, model parameter estimates (±SE) and doses (g a.i. ha^{-1}) required to provide 90% control (ED_{90}±SE) for 14 Belgian *C. esculentus* clones treated with bentazon and glyphosate when they were in the five true leaves stage (Experiment 2.1).

Table S3 Regression model, model parameter estimates (±SE) and doses (g a.i. ha^{-1}) required to provide 90% control ($ED_{90} \pm SE$) for 14 Belgian *C. esculentus* clones treated with preplant incorporated dimethenamid-P and S-metolachlor (Experiment 2.2).

Table S4 Regression model, model parameter estimates (±SE) and doses (g a.i. ha^{-1}) required to provide 90% control ($ED_{90} \pm SE$) for 14 Belgian *C. esculentus* clones treated with preplant incorporated dimethenamid-P and S-metolachlor (Experiment 2.2).

Table S5 Regression model, model parameter estimates (±SE) and doses (g a.i. ha^{-1}) required to provide 90% control ($ED_{90} \pm SE$) for 14 Belgian *C. esculentus* clones treated with preplant incorporated dimethenamid-P and S-metolachlor (Experiment 2.2).

Table S6 Regression model, model parameter estimates (±SE) and doses (g a.i. ha^{-1}) required to provide 90% control ($ED_{90} \pm SE$) for 14 Belgian *C. esculentus* clones treated with preplant incorporated dimethenamid-P and S-metolachlor (Experiment 2.2).

Table S7 Pearson's correlation coefficients between various growth characteristics (Experiment 1), and ED_{90} responses (Experiment 2) of a set of 14 Belgian *Cyperus esculentus* clones.

Table S8 Mantel test results for among-clone differences in herbicide response, and geographic distance matrices.