



PURE

Pesticide Use-and-risk Reduction in European farming systems with Integrated Pest Management

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**Collaborative Project
SEVENTH FRAMEWORK PROGRAMME**

D8.5

Report on the co-evolution of resistant and susceptible weed biotypes in herbicide-free field trials

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| Dissemination Level | |
| PU Public | PU |
| PP Restricted to other programme participants (including the Commission Services) | |
| RE Restricted to a group specified by the consortium (including the Commission Services) | |
| CO Confidential, only for members of the consortium (including the Commission Services) | |

1. Introduction

Herbicide-resistant (R) weed biotypes may evolve from herbicide-susceptible (S) biotypes when farmers rely too much on herbicides with the same mode of action. We hypothesised that when the trait of herbicide-resistance is gained, at the same time other traits would degrade (so-called 'fitness penalties'), so that evolution would favour biotype R over biotype S, only when the same type of herbicide remained in use.

To investigate this hypothesis we measured the essential life history traits of both R and S biotypes of two weed species: *Echinochloa crus-galli* and *Alopecurus myosuroides*. The experiments were carried out with biotypes from Italy and Denmark, respectively.

To investigate the importance of the measured differences in life history traits, we applied a generic model of annual weed population dynamics. Parameters for the different weed species and biotypes were estimated from our own experiments and from literature, and simulations were carried out to investigate the expected dynamics of the weeds under herbicide-free field situations.

The hypothesis for the long-term dynamics is that the S population would grow faster than the R population, because when no herbicides are applied, the R biotype would have no advantage of its herbicide resistance; it would only have the disadvantages of the accompanying fitness cost. If the fitness costs should result in a dramatic advantage of the S biotype over the R biotype, then herbicide-free cultivation (or, likewise, herbicide-based cultivation with an alternative mode of action) would be a method of reducing the R population and turning the wheel of herbicide-resistance evolution backwards.

2. Model description

The number of seeds in the soil seed bank (N_b ; m^{-2}) decrease with time due to the processes of seed mortality (μ ; y^{-1}) and seedling emergence (ε ; y^{-1}) and, increase due to net seed production (ΔP ; m^{-2}),

$$\Delta N_b = -(\mu + \varepsilon)N_b \Delta t + \Delta P$$

with a time step (Δt ; y) of one year.

The number of seedlings (N_s ; m^{-2}) equals the emergence,

$$N_s = \varepsilon N_b \Delta t$$

of which a certain proportion (σ ; y^{-1}) survives into mature plants (N_w ; m^{-2}),

$$N_w = \sigma N_s \Delta t$$

The above-ground weed biomass (M_w ; $g m^{-2}$) scales to weed density (N_w), taking intraspecific and interspecific competition with the crop (N_c ; m^{-2}) into account,

$$M_w = \frac{N_w}{b_0 + b_w N_w + b_c N_c}$$

with parameters for the inverse of biomass per weed plant at no competition ($b_0; \text{g}^{-1}$) and coefficients for intraspecific ($b_w; \text{g}^{-1} \text{m}^2$) and interspecific competition ($b_c; \text{g}^{-1} \text{m}^2$).

Weed seed production ($\Delta S; \text{m}^{-1}$) is proportional to weed biomass:

$$\Delta S = \frac{\rho M_w}{s}$$

with parameters for reproductive effort ($\rho; \text{g/g}$) and individual seed weight ($s; \text{g}$).

Of the seeds produced only a proportion ($\pi; \text{y}^{-1}$) will survive as the net seed production (ΔP) that enters the soil seed bank (N_s),

$$\Delta P = \pi \Delta S$$

3. Parameter estimation

Parameters were estimated for *Echinochloa crus-galli* (Table 1) and *Alopecurus myosuroides* (Table 2). We found fitness penalties in reproductive effort (ratio seed weight to total biomass) for *E. crus-galli* (reduced by 50% for the R biotype) and in emergence rate for *A. myosuroides* (reduced by 60% for the R biotype). Seedling survival was set low for both species and both biotypes (1-2%) reflecting the best possible control level.

4. Herbicide-free field scenarios

The model was implemented in R. Each simulation lasted 10 years starting out with an initial seed bank of 1 seed per m^2 . For those parameters estimated as an interval, a random value inside that interval was chosen before the simulation. The simulation was repeated 100 times, yielding 100 different outcomes due to the randomly chosen parameter values. The predicted weed population dynamics were summarized by quantile spline regressions (10%, 50%, 90%) (*qsreg* of the *fields* R package).

For *E. crus-galli*, the fitness penalty in reproduction resulted in a stable population density with a population growth rate close to zero (Fig. 1), whereas for *A. myosuroides*, the fitness penalty in emergence rate resulted in no discernible difference in population growth rate between the two biotypes (Fig. 2).

5. Conclusion

In a herbicide-free field situation, the S and R biotypes of the two species behaved differently. For *E. crus-galli*, the R biotype remained about constant in density, whereas the S biotype increased markedly in density. So, for this species the R biotype would make up a still smaller proportion of the total *E. crus-galli* population. The population growth rate close to zero for the *E. crus-galli* R biotype relies on an effective control (98%- 99% in the model). This will be difficult (or, at least, costly) to achieve without herbicides. Alternatively, other herbicides to which the R biotype is still susceptible could be used. A more diverse crop rotation (continuous maize in the model) would likewise help to lower the population increase of both the S and R biotype.

Table 1. Model parameter estimates for *Echinochloa crus-galli* (herbicide-susceptible) in continuous maize. S: herbicide-susceptible; R: herbicide-resistant. [a;b]: interval of values.

| Parameter | Value | Source |
|--|-------------------|---------------------------|
| Seed bank mortality (μ ; y^{-1}) | [0.7; 0.8] | Egley and Chandler (1983) |
| Seedling emergence (ε ; y^{-1}) | [0.05; 0.1] | CNR experts |
| Seedling survival (σ ; y^{-1}) | [0.01; 0.02] | Chosen control efficacy |
| Inverse of plant weight without competition (b_0 ; g^{-1}) | 0.0006 | CNR experiments |
| Intraspecific competition (b_w ; $g^{-1} m^2$) | 0.0004 | CNR experiments |
| Interspecific competition (b_c ; $g^{-1} m^2$) | [0.005; 0.0015] | Workshop experts |
| Reproductive effort (ρ ; g/g) | S:0.26; R: 0.13 | CNR experiments |
| Individual seed weight (s ; g) | [0.0015 ; 0.0020] | CNR experiments |
| Survival of new seeds (π ; y^{-1}) | [0.7 ; 1] | Workshop experts |

Table 2. Model parameter estimates for *Alopecurus myosuroides* in continuous winter wheat. S: herbicide-susceptible; R: herbicide-resistant.

| Parameter | Value | Source |
|--|---------------------------------|---|
| Seed bank mortality (μ ; y^{-1}) | 0.7 | Moss (1990) |
| Seedling emergence (ε ; y^{-1}) | S: [0.15; 0.30]; R: [0.09;0.18] | Moss (1990) |
| Seedling survival (σ ; y^{-1}) | [0.01; 0.02] | Chosen control efficacy |
| Inverse of plant weight without competition (b_0 ; g^{-1}) | 0.033 | Chauvel et al. (2005), Keshtkar et al. (2015) |
| Intraspecific competition (b_w ; $g^{-1} m^2$) | 0.0057 | Wilson and Wright (1990) |
| Interspecific competition (b_c ; $g^{-1} m^2$) | 0.0002 | Keshtkar et al. (2015) |
| Reproductive effort (ρ ; g/g) | 0.40 | Chauvel et al. (2005) |
| Individual seed weight (s ; g) | 0.0016 | Chauvel et al. (2005) |
| Survival of new seeds (π ; y^{-1}) | 0.45 | Moss (1990) |

For *A. myosuroides*, herbicide-free cultivation would not help to control the R biotype. Even if the farmer completely abandoned herbicides (or just the types of herbicides that the R biotype had become resistant against), the R biotype together with the S biotype would remain serious weeds in the field. Slight changes in the relative population densities of the R and S biotypes might evolve with time but not to any degree that this would eradicate the R biotype. Long-term, herbicide-free field trials are very unlikely to change this conclusion, at least for this species and biotype.

Even before the advent of herbicide-resistance, farmers had to manage many different weed species. These were controlled in different ways according to their biology. Problematic weeds were controlled by diversifying the crop rotation and by diversifying the control measures (chemical, non-chemical, direct and indirect). In this perspective, an R biotype should be seen as yet another weed species – with the same options for control as the S biotype, except for a restriction in the applicable herbicide spectrum.

A joint scientific publication on this work is being written by researchers from AU, CNR and WU.

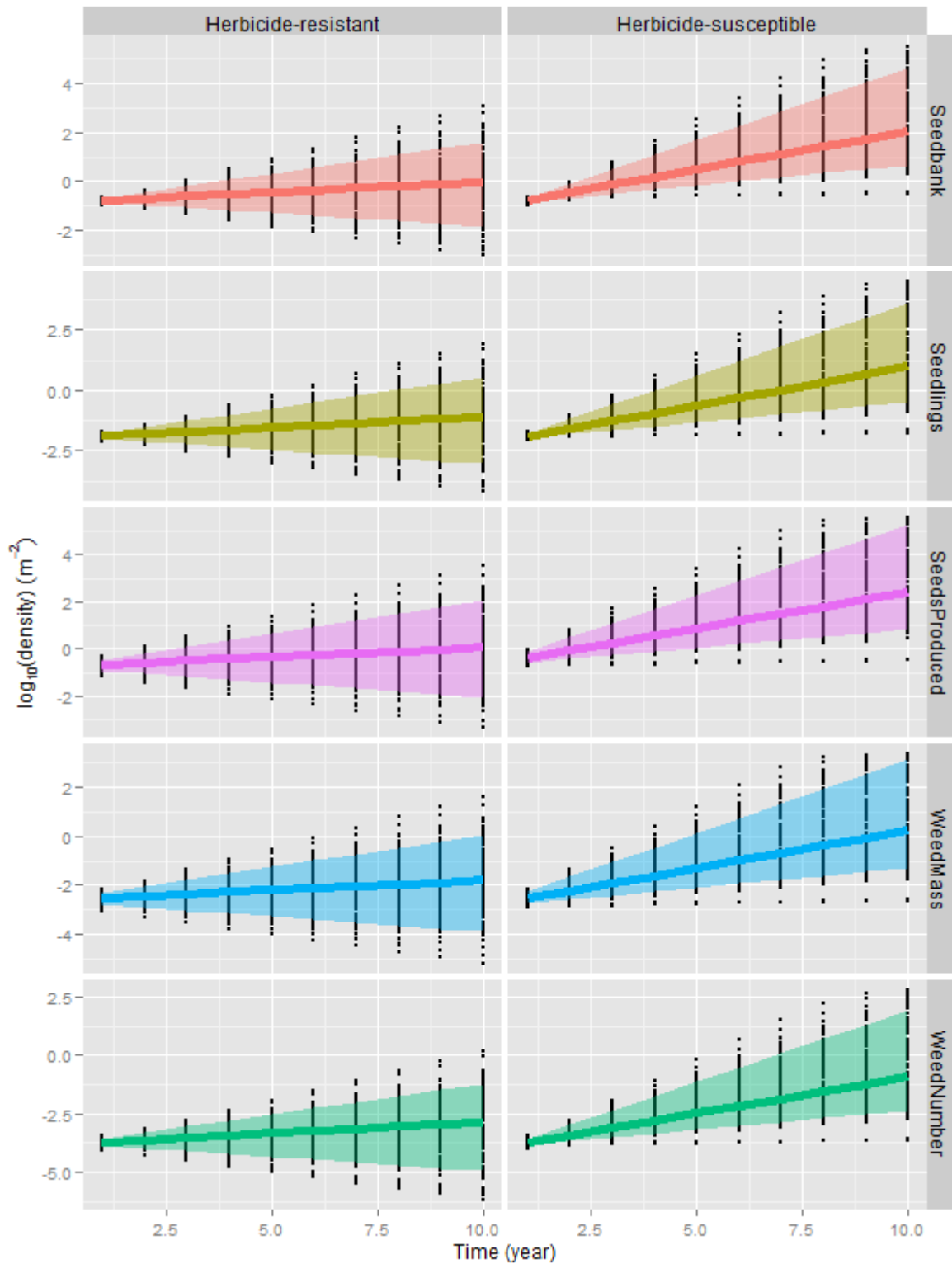


Fig. 1. Predicted population dynamics of two different *Echinochloa crus-galli* biotypes (herbicide-resistant and herbicide-susceptible) from a starting population of 1 seed per m^{-2} .

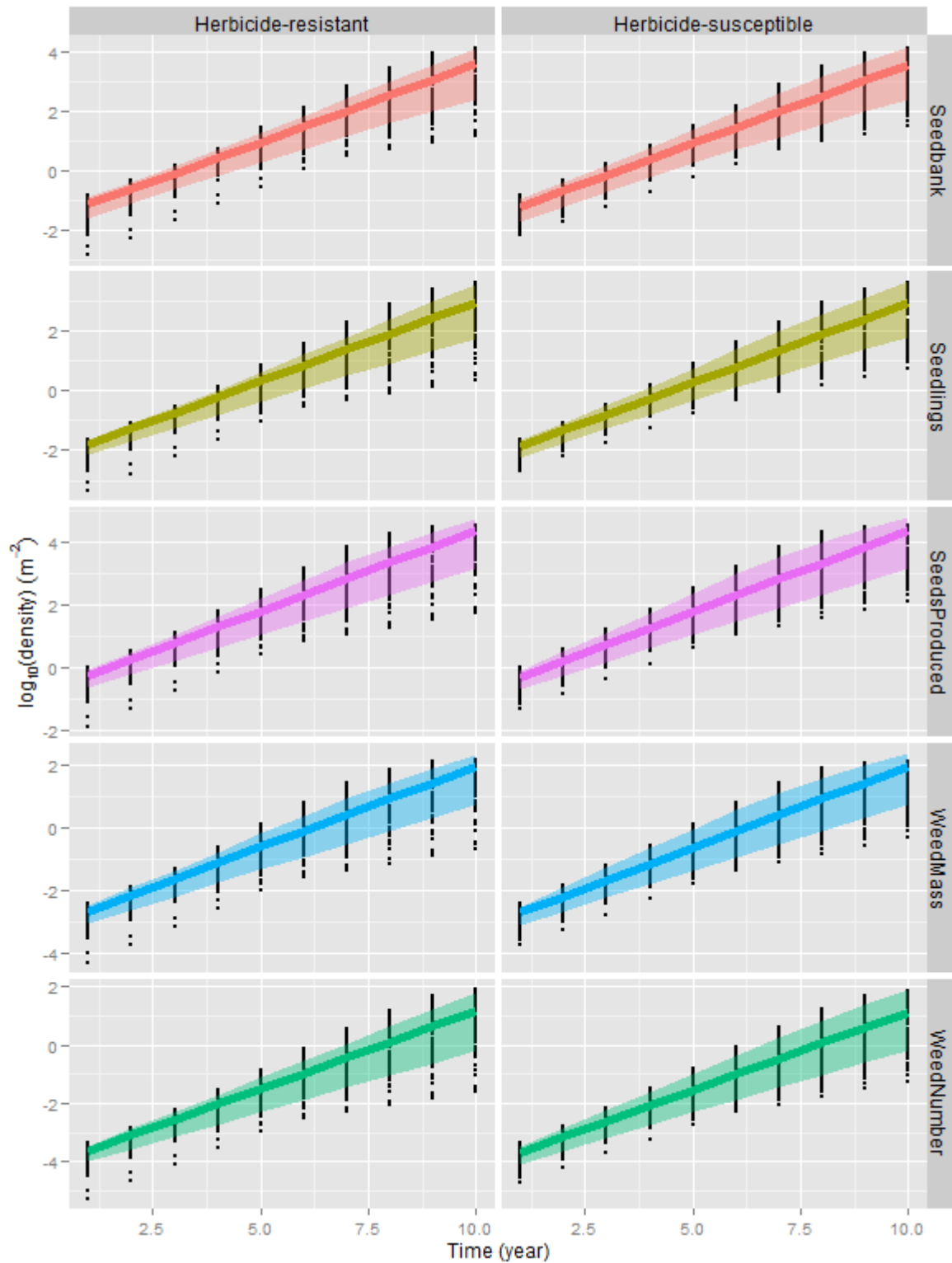


Fig. 2. Predicted population dynamics of two different *Alopecurus myosuroides* biotypes (herbicide-resistant and herbicide-susceptible) from a starting population of 1 seed per m^{-2} .

6. Literature

Chauvel, B., Guillemain, J.P., Letouze, A., 2005. Effect of intra-specific competition on development and growth of *Alopecurus myosuroides* Hudson. *European Journal of Agronomy* 22, 301-308.

Egley, G.H., Chandler, J.M., 1983. Longevity of weed seeds after 5.5 years in the Stoneville 50-year buried seed study. *Weed Science* 31, 264-270.

Keshtkar, E., Mathiassen, S., Kudsk, P., 2015. No ecological fitness cost of two acetyl-coenzyme A carboxylase non-target site resistant *Alopecurus myosuroides* Huds populations: vegetative and reproductive output under normal and biotic stressful condition. *Submitted*.

Moss, S.R., 1990. The seed cycle of *Alopecurus myosuroides* in winter cereals: a quantitative analysis, Proceedings of the EWRS Symposium on Integrated Weed Management in Cereals, 4-6 June 1990, Helsinki, Finland, pp. 27-35.

Wilson, B.J., Wright, K.J., 1990. Predicting the growth and competitive effects of annual weeds in wheat. *Weed Research* 30 201-211.

7. Appendix

The model source code in R.

```
### Prepare R environment
rm(list=ls(all=TRUE))
graphics.off()
library(fields)
library(ggplot2)
library(plyr)
library(reshape2)
setwd("C:/data/RDev/pure_weed_model") # Change this according to your working folder

# Overwrite global names
s = {}
pi = {}

# Data frame of parameter values
parameters = data.frame()

# Set parameter values, fixed or stochastic
draw_ecg = function() {
  # All parameters are first S and then R
  # Crop density
  Nc <- rep(7,2) #per m2

  # Initial seed bank density
  NbInit <- rep(1,2) #per m2

  # Seed bank proportional loss per year
  mu <- runif(2, 0.7, 0.8)

  # Seed bank proportional emergence per year
  eps <- runif(2, 0.05, 0.1)

  # Seedling survivorship
  sigma <- runif(2, 0.01, 0.02)
```

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```
### Weed biomass coefficients
# Compensation of delayed emergence
k = 50

# Inverse of final biomass of one plant without competition
b0 <- rep(0.0006,2) #plant/g

# Intraspecific competition
bw <- rep(0.0004,2) # g/m2

# Interspecific competition
bc <- runif(2, 0.0001, 0.0003)*k # g/m2

### Seed production
# Reproductive effort
rho <- c(0.26, 0.13) #g/g

# Weight of one seed
s <- runif(2, 0.0015, 0.0020) #g

# Seed survival
pi <- runif(2, 0.7, 1)

# Collect parameter values
parameters <- rbind(parameters, data.frame(eps, sigma, rho, s, pi))
}

draw_am = function() {
  # All parameters are first S and then R
  # Crop density
  Nc <- rep(400,2) #per m2

  # Initial seed bank density
  NbInit <- rep(1,2) #per m2

  # Seed bank proportional loss per year
  mu <- rep(0.7,2)

  # Seed bank proportional emergence per year
  k = 0.6 # reduced emergence of R biotype

  eps <- runif(2, 0.15, 0.3)
  eps[2] = eps[2]*k

  # Seedling survivorship
  sigma <- runif(2, 0.01, 0.02)

  ### Weed biomass coefficients
  # Inverse of final biomass of one plant without competition
  b0 <- rep(0.033,2) #plant/g

  # Intraspecific competition
  bw <- rep(0.0057,2) # g/m2

  # Interspecific competition
  bc <- rep(0.0002,2) # g/m2

  ### Seed production
  # Reproductive effort
  rho <- rep(0.4,2) #g/g

  # Weight of one seed
  s <- rep(0.0016,2) #g
```


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```
# Seed survival
pi <- rep(0.45,2)

# Collect parameter values
parameters <- rbind(parameters, data.frame(eps, sigma, rho, s, pi))
}

### Model

DeltaNb = function(Nb, DeltaP) {
  - (mu + eps)*Nb + DeltaP
}

Ns = function(Nb) {
  eps*Nb
}

Nw = function(Ns) {
  sigma*Ns
}

Mw = function(Nw) {
  Nw/(b0 + bw*Nw + bc*Nc)
}

DeltaS = function(Mw) {
  rho*Mw/s
}

DeltaP = function(DeltaS) {
  pi*DeltaS
}

### Figures

figure = function(sim) {
  # Quantile regression on predictions
  pred = ddply(sim, .(variable),
    function(x) {
      model1 = qsreg(x$Year, log10(x$value), alpha=0.1)
      model2 = qsreg(x$Year, log10(x$value), alpha=0.5)
      model3 = qsreg(x$Year, log10(x$value), alpha=0.9)
      data.frame(Year=x$Year, Variable=x$Variable, Biotype = x$Biotype, Pred1=predict(model1,
x$Year), Pred2=predict(model2, x$Year), Pred3=predict(model3, x$Year) )
    }
  )
  plot = function(titl) {
    ggplot(sim, aes(x=Year, y=log10(value))) +
      geom_point(size=1) +
      geom_smooth(data=pred, aes(x=Year, ymin=Pred1, y=Pred2, ymax=Pred3, colour=Variable,
fill=Variable), size=2, stat="identity") +
      theme(legend.position="none") +
      labs(title=titl, x="Time (year)", y=expression(paste(log[10], "(density) ", (m^-2), "\n",
sep=""))) +
      theme(strip.text=element_text(size=12)) +
      facet_grid(Variable~Biotype, scales = "free_y")
  }

  png(paste0(species, ".png"), 600,800)
  print(plot(""))
  dev.off()
}
```

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```

windows(8,15)
print(plot(paste0("Simulated dynamics - ",species,"\n")))
}

### Simulation

run_once = function(duration) {
  if (species=="ecg") draw_ecg() else draw_am()
  NbY = NbInit
  DeltaPY = 0
  result = {}
  for (year in 1:duration) {
    NbY = NbY + DeltaNb(NbY, DeltaPY)
    NsY = Ns(NbY)
    NwY = Nw(NsY)
    MwY = Mw(NwY)
    DeltaSY = DeltaS(MwY)
    DeltaPY = DeltaP(DeltaSY)
    state = c(year, NbY, NsY, NwY, MwY, DeltaSY)
    result = rbind(result, state)
  }
  result = as.data.frame(result)
  sr = function(x) paste0(x, c("S", "R"))
  colnames(result) = c("Year", sr("Seedbank"), sr("Seedlings"), sr("WeedNumber"), sr("WeedMass"),
sr("SeedsProduced"))
  rownames(result) = {}
  result
}

run_many = function() {
  num_years = 10
  num_replicates = 100
  sim = lapply(rep(num_years, num_replicates), run_once)
  sim = ldply(sim)
  sim$Rep = factor(sort(rep(1:num_replicates, num_years)))
  sim0 = sim
  sim = melt(sim, id.vars=c("Year", "Rep"))

  last = function(x) {
    y = as.character(x)
    substr(y, nchar(y), nchar(y))
  }
  first = function(x) {
    y = as.character(x)
    substr(y, 1, nchar(y)-1)
  }
  sim$Variable = first(sim$variable)
  sim$Biotype = last(sim$variable)
  sim$Biotype[sim$Biotype=="S"] = "Herbicide-susceptible"
  sim$Biotype[sim$Biotype=="R"] = "Herbicide-resistant"

  figure(sim)
}

### Main
species = "ecg"
run_many()
species = "am"
run_many()

```