PROJECT DISCOVERING ASSOCIATION (GROUP 8) FINAL REPORT

THE BLACK TULIP PROJECT

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ABSTRACT

The aim of this "Discovering Association" project was to set up an experimental design to answer a research question, to obtain a sample size for the experiment (possibly based on a pilot study), to conduct an exploratory phase of the data to get as much insight from the data as possible, to run statistical analysis to address the research question and to discuss and interpret the results to finally draw conclusions. Associations related to repeated measures over time were considered and dealt with a GEE approach.

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1 INTRODUCTION

1.1 The Black Tulip Project

WHY A STUDY ON THE BLACK TULIP ?

For specific reasons explained in Bijnens (2016) [1], it was of primary interest to determine how cut black tulip flowers from a remote island could be best processed for shipment and sustained fresh for a period of time longer than the required journey to deliver them to France.

THE PROBLEM

Black tulips grew only locally in a remote island and had to be cut. No bulb removal or seed collection was allowed. The cut flowers had to be processed for shipment and delivered fresh to destination. The shipment and ocean's journey took between 8 and 22 days depending on ocean's conditions. The problem was that the number of days a cut black tulip could naturally stay fresh and in good shape was shorter than the average journey time.

A WAY TO A SOLUTION

- It was proposed to find out and conduct conditioning/preservation methods to sustain freshness time of *similar* flowers.
- It was proposed to use one appropriate chemical additive to extend flowers freshness time.
- Fifteen chemical additives (including distilled water) were available for trials.
- It was of primary interest to validate or unvalidate the effect of these individual chemical additives on flower sustaining longer time.

2 SCIENTIFIC QUESTION

2.1 Research Question

Test and determine whether or not one of the 14 chemical compounds has a positive effect on the time that a flower stays fresh.

WHAT ARE THE SATISFYING CONDITION AND SPECIFICATION OF THE MEASURE OF SUCCESS ?

- The study objective is met (success) if a compound is found to increase the time the flower stays fresh when compared to distilled water alone.
- The quantitative measure of success is a 10% increase in time of staying fresh when compared to water alone (The size effect that we wanted to be able to detect, *if there were truly an effect*, was 10% in increase in time a flower stays fresh).

3 POPULATION, SOURCE OF VARIABILITY AND EXPERIMENTAL UNITS

POPULATION AND SURROGATE POPULATION

The target population is made of Black Tulip flowers. These flowers belong to the botanic family of *Liliaceae*, in the genus *Tulipa* and the species *Black Tulip* sp. The Black Tulip flowers are the specimens of real interest.

The surrogate population encompasses flowers belonging to the botanic family of *Rosaceae*, in the genus *Rosa* with two species : *Floribunda* sp. and *Hybrid Tea* sp.

WHAT ARE THE SOURCES OF VARIABILITY ?

The following sources of variability were considered :

1. Variability within flowers was expected as well as variability due to flower conditioning and due to chemical additive processing (treatment).
2. Differences in flower species of the same genus and family was a possible source of variability.
3. Differences between plot locations were a possible source of variability due to sunlight or meteorological privileged exposition or soil composition.
4. Soil inhomogeneity within a plot location was a possible source of variability.
5. Variability due to operators or raters was not considered because all the manipulations were planned to be done by a single unique operator.

WHAT ARE THE EXPERIMENTAL UNITS ?

We were constrained not to use experimental units from the population of Black Tulip flowers in the remote island but had to use instead experimental units taken from a surrogate population of flowers of 2 different species supposed similar to the specimen of real interest. The experimental units were flowers belonging to the *Rosacea* family, genus *Rosa* species *Floribunda* sp. and *Hybrid Tea* sp. to be grown in France, in the gardens of Jean-Baptiste.

4 DISCLAIMERS AND ASSUMPTIONS

4.1 Disclaimers

Liability is disclaimed on any risk related to these two constrained experimental conditions :

1. The candidate chemical compounds were not tested directly on the specimens of real interest (*Black Tulip* sp.) but on specimens of a surrogate population (*Rosa Floribunda* sp. and *Rosa Hybrid Tea* sp.).
2. The black color results in the absence of any component absorbing light of any visible wavelength whereas the experimental units have extended visible wavelength absorption spectra. The effect of the chemical compound on black color was not measured in the study.

Conclusions drawn from the experimental material might not be applicable to the Black Tulip.

4.2 Assumptions

For inference purposes and for the setting of the experimental design, the following assumptions were taken :

1. Soil and meteorological different conditions between the island and the experimental plots were supposed to have no effect on the response variables.
2. No interaction with third party biological species (birds, snails, worms, insects, fungi and bacteria) was assumed.
3. A single unique operator/rater was on duty for the experimental work and data collection. This was made possible due to the blooming time lag between the two experimental flower species.
4. Soil inhomogeneity within a plot location was controlled (blocked) by randomizing evenly the subplots to the assigned chemical additives.
5. Differences between species due to soil differences within plots were controlled (blocked) by randomizing evenly the subplots between the two experimental species.
6. The data collection protocol was considered reliable and no missing values had been anticipated or taken into account for sample size calculation (no security factor for missing values had been considered).

5 REQUESTED SAMPLE SIZE

5.1 Information required for the sample size calculation

To determine a sample size (N, i.e. the number of flowers required as experimental units), 4 inputs are needed (α, β, δ, σ^2) and an underlying statistical model is to be specified :

1. The type I error (risk of false positive or risk of erroneous concluding to a an effect although there were none) was fixed to $\alpha = 0.05$.
2. The type II error (risk of false negative or the risk of not being able to detect an effect although there were a real effect) was fixed to $\beta = 0.20$. Equivalently, the power of the design is $1 - \beta = 0.80$. If there is an effect, our probability to be able to detect it was fixed upfront at 80%.
3. $\delta = 10\%$, was the size effect we fixed as specification of our measure of success in the research question. If we found a compound that would increase the number of days that a flower stays fresh of 10% at least, as compared to distilled water, the result of the black tulip project would be a success.
4. σ^2, is the inherent variability in the number of days a flower stays fresh in the population of our experimental units. This was unknown and motivated the need of a pilot study to get an estimate of the variability of the surrogate population.
5. The proposed statistical model used for sample size calculation was Poisson regression because a possible response variable we were dealing with is a count (count of the number of days a flower stays fresh). It is worth indicating to the reader that logistic regression could also be considered if the response is seen as a binary outcome for the freshness status of a flower at a given day point. As the Bernoulli and the Poisson distribution are both discrete distributions, belong both to the same exponential family, and most importantly because of the Poisson theorem states that a binomial distribution has a Poisson distribution as a limit when both the sample size is large ($n \to \infty$) and the product of the sample size with the Bernoulli parameter π (i.e. $n \cdot \pi \to \lambda$) is not too large, the sample size result can be considered as applicable to both statistical models (Poisson or logistic regression) as a first approximation.

5.2 Pilot Study

A pilot study was conducted in order to obtain estimates of variance for sample size calculation. It consisted of 20 flowers of each species grown in the northern garden. The freshness outcome of those cut flowers was monitored over time when preserved in distilled water only. A dataset "Pilot_Group8.txt" with the results was provided by Bijnens and Vandendijk (2016a)[2].

5.2.1 *Model setting prior to sample size estimation*

A multiple Poisson regression model was assumed. The number of days a flower stayed fresh was the response variable while compound at 15 levels, flowers species at 2 levels (Floribunda/Hybrid Tea) and garden at 2 levels (Northern/Southern) were included as predictor variables (covariates) in the proposed model. With Y_i, the count number indicating the number of days the i^{th} flower stays fresh, the model is given by :

$$Y_i \sim \text{Poisson}\,(\lambda_i) \quad (1)$$

$$Ln\,(\lambda_i) = \beta_0 + \sum_{n=1}^{15} \beta_n \cdot X_{in} + \beta_{16} \cdot S_{i16} + \beta_{17} \cdot G_{i17} \quad (2)$$

INDICATOR VARIABLES :

$$X_{in} = \begin{cases} 1 & \texttt{if compound=}\ n \in [2, \ldots, 15] \\ 0 & \texttt{if compound=}\ 1\,(\texttt{distilled water}) \end{cases}$$

$$S_{i16} = \begin{cases} 1 & \texttt{if species type=}\ 1\,(\texttt{Floribunda}) \\ 0 & \texttt{if species type=}\ 2\,(\texttt{Hybrid Tea}) \end{cases}$$

$$G_{i17} = \begin{cases} 1 & \texttt{if garden=}\ 1\,(\texttt{Northern}) \\ 0 & \texttt{if garden=}\ 2\,(\texttt{Southern}) \end{cases}$$

5.2.2 *Variance estimation of the surrogate population*

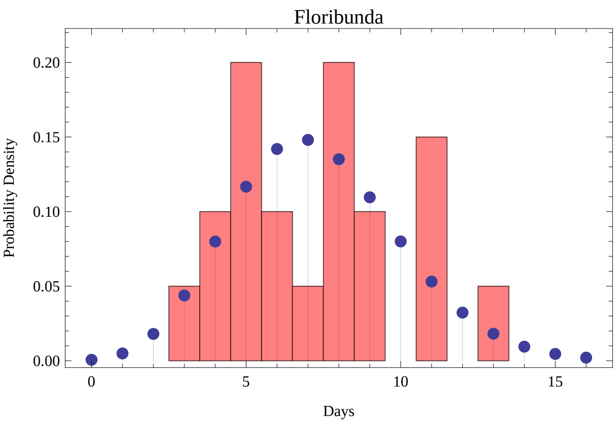

Figure 1: Observed Histogram and Poisson probability for days count, *Floribunda* sp.

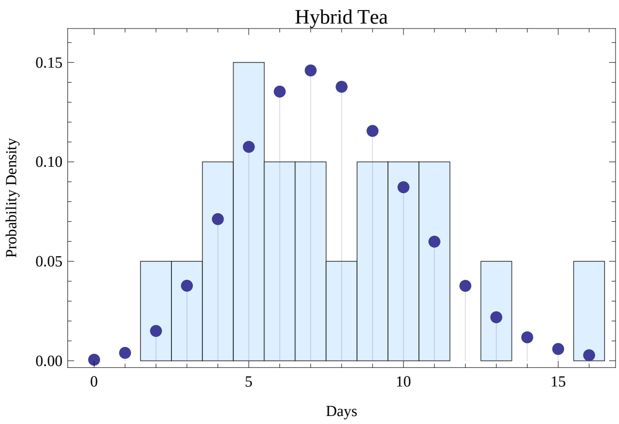

Figure 2: Observed Histogram and Poisson probability for days count, *Hybrid Tea* sp.

The pilot data were explored in order to have an indication of the variability of the surrogate population we were dealing with. The histograms of the observed frequency for the number of days the flowers stayed fresh in both species are displayed on Figure 1 and Figure 2 with the fitted Poisson distribution (filled points). The results for the mean and the variance for the two species are tabulated in Table 1. For Poisson distribution the mean is equal to the variance. A F test for the equality of the two variances

Table 1: Pilot data summary statistics and variability estimates.

	Floribunda sp. $S_{16} = 1$	*Hybrid Tea* sp. $S_{16} = 0$
Mean (days) λ	7.30	7.695
Variance $\sigma^2 = \lambda$	7.55	12.787

showed that the variances for the two species were not significantly different from each other (F statistics $= 1.69 < F_{0.975,19,19} = 2.526$, p-value$= 0.1308$). The 95% confidence interval for the variance ratio to be equal to one is $[0.66 \longleftrightarrow 4.20]$. The variance to the mean ratio is 1.03 for *Floribunda* sp. and 1.66 for *Hybrid Tea* sp. Both are included in the confidence interval. We concluded that the distribution of the response variable conformed to the underlying Poisson distribution and from the confidence interval of the variance ratio that there are no indication of overdispersion. We took, for our sample size calculation, the variance value (also equal to the mean) that lead to the higher sample size (conservative) : $\sigma^2 = \lambda_0 = e^{\beta_0} = 7.30$.

5.3 Sample Size Calculation

For one compound compared to water, the effect size we are interested in, all other covariates held constant, is expressed by :

$$\lambda_0 = e^{\beta_0} \text{ (for water)} \tag{3}$$
$$\lambda_1 = e^{\beta_0+\beta_1} = e^{\beta_0} \cdot e^{\beta_1} \text{ (for the compound of interest)} \tag{4}$$

The requested effect size threshold of 10% means that :

$$\delta = \frac{\lambda_1}{\lambda_0} = e^{\beta_1} = 1.1 \tag{5}$$

With $\lambda_0 = 7.30$, we have $\lambda_1 = 7.30 \times 1.1 = 8.03$ (10% for effect size) and given our previous adopted assumptions $\alpha = 0.05$, $1 - \beta = 0.80$, the sample size calculation relies on the hypothesis test for equality to zero of the β_1 parameter in the Poisson regression model :

NULL HYPOTHESIS AND ALTERNATIVE :

$H_0 : \beta_1 = 0$
$H_a : \beta_1 > 0$
The sample size was determined with the **one sided** z test with G∗Power following Signorini (1991)[4] and Faul *et.al* (2009)[5] or SAS with an iterative procedure. The test is one sided because we are interested only in the best performing compound when compared to distilled water. The significance level $\alpha = 0.05$ was not divided by 14 as we are not interested in multiple comparisons but were looking for just one compound (the best). The relevant SAS code #1 is given in appendix. Both G∗Power and SAS gave the same results presented in table 2. We concluded that we needed 178 flowers at least per compound arm, rounded to 180. The adopted total sample size for the Tulip Project was 180×15 arms $= 2700$ flowers.

Table 2: Sample size results from G∗Power and SAS

	G∗Power ONE SIDED	SAS ONE SIDED
Sample Size per arm (N)	178	178
True α	0.05	0.05
Setting α	0.05	0.10
$1-\beta$	0.80	0.80
Effect Size (10%)	1.1	
λ_0	7.30	7.30
λ_1	8.03	8.03

6 EXPERIMENTAL DESIGN

6.1 Rationale of the experiment

The rationale of the experiment was to test on a sample of the surrogate population the effect of each of the 14 compounds taken separately and to compare to a control (distilled water). There were 14 treatment arms in parallel.

We carried out on a number $N = 2700$ (total sample size) of individual flowers the same sequence of actions we would have done on the Black Tulip :

- Cut the flower in the right plot.
- Immerse the flower stem in water.
- Add assigned compound (experimental factor) on day point 0 and condition the flower according to the protocol.
- Conduct daily measures for a minimum of 22 days or until the flower had faded.

The same number ($\frac{N}{15} = 180$) of subjects were assigned to one treatment arm and were daily examined by a single unique rater during minimum 22 days or till fading. A reproducible decision rule was followed by the rater to declare if the flower was fresh or not.

WHAT WAS MEASURED ? The following quantitative flowers attributes were measured :

1. The flower diameter (a 10% reduction from day 0 results in declaring the flower not fresh).
2. The daily loss of petals and leaves (the loss of more than 2 petals from day 0 results in declaring the flower not fresh).
3. The stem bending angle (a change of more than 15 degrees from day 0 results in declaring the flower not fresh).
4. The location in the color scale unit (a change of more than one color unit in the color scale results in declaring the flower not fresh).

6.1.1 *What is the response variable ?*

All the previous measures were combined in a binary outcome (yes/no the flower is still fresh). The response variable were the daily records of the binary outcome (1/0 : the flower is fresh). A 1 is a fresh flower. A 0 is a non fresh flower. The daily binary records were repeated measures over discrete time points. From these daily binary records we could also have the count of the number of days the flower stayed fresh. For the Poisson regression model, the count of the number of days the flower stayed fresh was used. For the logistic regression model, the daily binary outcome records were used.

6.1.2 *The split plot randomized blocks*

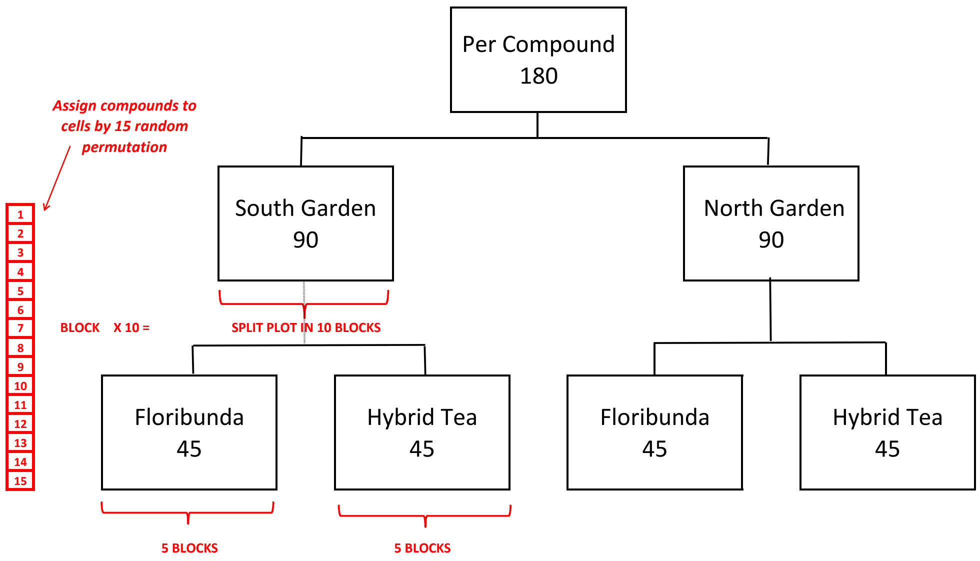

Figure 3: Sample size splitting and randomization.

The setting of our experimental design, very popular in agronomy since its introduction by Fisher (1935) [6], was the split plot randomized blocks. It served two purposes :

1. We were blocking two factors :
   - Garden (first level of the split) : each garden was split in the same number of blocks (10 blocks) of the same size.
   - Species (second level of the split): each species was evenly distributed between the blocks in each garden (5 blocks with *Floribunda* sp. and 5 blocks with *Hybrid Tea* sp.).

   Each block was subdivided in 15 cells of the same size where the 15 compounds were assigned by random permutations so that each block had the 15 compounds and so that the flowers assigned to a given compound would not come from the same place (see on Figure 4 for instance the flowers from the north garden assigned to treatment 7 came from the yellow cells).
2. Bias and systematic error (for example due to edge effects or privileged sun exposition or due to soil composition inhomogeneity within the garden) was minimized.

Figure 3 shows the 2 levels of the split with the blocked factors Garden and Species (=Type). Figure 4 shows how the assignment of the compounds was randomly permuted within a block. Note that each block always had 15 cells with a different compound in each cell. Each cell had 9 flowers. Each block had 135 flowers. Each compound was assigned to 90 flowers from each garden (45 *Floribunda* sp. and 45 *Hybrid Tea* sp.). Each garden had 1350 flowers and the total sample size was 2700 flowers.

	1	2	3	4	5	6	7	8	9	10
1	4	2	5	9	14	11	13	7	11	4
2	15	13	1	3	4	6	9	11	5	14
3	10	1	3	15	8	12	12	4	13	8
4	7	11	12	7	10	14	7	15	7	6
5	5	10	4	8	12	4	14	2	10	5
6	8	3	15	12	13	9	6	12	4	12
7	14	4	10	2	3	7	11	1	1	13
8	9	9	2	13	1	10	3	14	2	11
9	3	12	8	10	7	1	2	6	12	2
10	1	7	9	11	2	3	5	5	9	7
11	13	8	6	6	15	8	15	10	15	10
12	2	14	13	1	9	5	4	8	8	1
13	12	15	14	4	5	15	1	3	14	3
14	6	6	11	14	6	13	8	9	6	15
15	11	5	7	5	11	2	10	13	3	9

Figure 4: Split Plot Randomized Blocks in the North Garden.

7 STATISTICAL MODELS

The required sample size, calculated with a Poisson regression specified model also provided enough power to detect the desired effect size under logistic regression. Both models lead to the same conclusions with respect to the research question. The compound effect, species effect and the garden effect were estimated with both statistical models. Two different GEE approaches were conducted for the statistical analysis depending on the model.

7.1 Poisson regression and GEE approach

With the Poisson regression, the response is the count of the number of days a particular flower stays fresh. There is no repeated measures over time as we counted the time in days as a global response for each flower. However, we assumed a working correlation structure (compound symetry or exchangeability) to incorporate the expected (in)dependence *within* the blocks resulting from the randomized split plot and to check that there was no block effect possibly related to an underlying soil composition gradient or a systematic privileged meteorological exposure *within* blocks. Although no formal inference can be drawn from the fitted correlation matrix, we will retro-check the plausibility of our working correlation assumption by comparing the empirical estimates with the model base estimates, under both the exchangeable and the independent correlation structure. Besides, we formally tested for the absence of effect *between* blocks through the incorporated `block` as an extra covariate in the Poisson regression model.

7.1.1 *Poisson Regression Model*

A block covariate (with 10 levels) was added to the Poisson regression modeling equation 2 in order to incorporate the randomized split plot design in our statistical analysis. This block extra term incorporation was also motivated by the fact the block information was available in the Black Tulip Study dataset provided by Vandendijck et al.(2016b) [3]. Hence, equation 2 became (with the same indicator variables as before plus a covariate with 10 levels $B_{i\,q}$) :

$$\mathrm{Ln}\,(\lambda_{i(q)}) = \beta_0 + \sum_{n=1}^{15} \beta_n \cdot X_{in} + \beta_{16} \cdot S_{i16} + \beta_{17} \cdot G_{i17} + \beta_{20} \cdot B_{i\,q} \quad (6)$$

ADDED BLOCK COVARIATE :

$$B_{i\,q} = q \in [1, \ldots, 10]$$

7.1.2 *GEE with exchangeable working correlation structure*

We wanted to investigate for a possible *within* block effect (possibly due to a soil effect within block for instance). We needed to find a way to develop a model that could account for the correlation between flowers belonging to the same block. This requires the knowledge of a true correlation matrix for the flowers within a block. We do not know *a priori* this matrix but to alleviate the problem, following the GEE methodology, we specify an approximation of the true matrix instead. The approximated covariance matrix, called working covariance matrix, was chosen in the form of the exchangeable structure. It should be kept in mind that GEE methods consistently estimate the regression parameters (β's) even if one misspecifies the correlation structure. An estimate of the covariance matrix can be constructed that will be asymptotically consistent despite the wrong choice of the working correlation structure. The adopted assumption was:

EXCHANGEABLE working correlation assumption (q is the block index) :

$$\mathrm{Corr}(Y_{i,q}, Y_{j,q}) = \begin{cases} 1 & (i = j) \\ \rho & (i \neq j) \end{cases} \quad (7)$$

$$\rho \stackrel{?}{=} 0 \quad (8)$$

If there were no effect within a block, the outcome of a flower in a block would be independent of the outcome of any other flower in the same block unless due to a different compound effect. Because the compounds were randomly permutated within a block, we should expect that $\rho = 0$. It should be noted that, with 10 blocks per garden, the dimension of the working correlation matrix is a 10 x 10 matrix in a single garden but a 20 x 20 dimension matrix as the blocks are nested in 2 gardens (north and south). It must also be highlighted that in the nested case, a garden effect should not be confused with a block effect : our assumption was that there was no expected block effect within a garden.

7.2 Logistic regression and GEE approach

Logistic regression with GEE was used for further and complementary analysis. A GEE approach was conducted to take the correlation structure between the outcomes of a flower due to the repeated measure of the outcome over time (same flower across successive day time points). A first order auto-regressive, i.e. AR(1), was applied as the working assumption for the correlation structure due to the longitudinal nature of the repeated measurements. The longer the number of days between two outcomes, the smaller the correlation between the two outcomes for the same flower.

7.2.1 *Logistic Regression Model*

Let Y_{ij} be the binary outcome indicating the freshness status of a flower where the indices are

- i is the i^{th} flower
- j is the j^{th} day (in a set of 30 days after day 0)

A multiple logistic regression model was assumed, with linear time trends, for the 14 treatments separately (to be compared with distilled water), taking into account the flower species (Floribunda/Hybrid Tea) and the Garden (North/South). The model was given by :

$$Y_{ij} \sim \text{Bernoulli}(\pi_{ij}) \tag{9}$$

$$\begin{aligned} \text{logit}(\pi_{ij}) &= \beta_0 + \sum_{n=1}^{15} \beta_n \cdot X_{in} + \beta_{16} \cdot S_{i16} + \beta_{17} \cdot G_{i17} + \beta_{18} \cdot t_{ij} \\ &\quad + \sum_{n=1}^{15} \beta_{18+n} \cdot X_{i18+n} \cdot t_{ij} \end{aligned} \tag{10}$$

INDICATOR VARIABLES :

$$X_{i\,n} = X_{i\,18+n} = \begin{cases} 1 & \texttt{if compound=}\ n \in [2, \ldots, 15] \\ 0 & \texttt{if compound=}\ 1\ (\texttt{distilled water}) \end{cases}$$

$$S_{i16} = \begin{cases} 1 & \texttt{if species type=}\ 1\ (\texttt{Floribunda}) \\ 0 & \texttt{if species type=}\ 2\ (\texttt{Hybrid Tea}) \end{cases}$$

$$G_{i17} = \begin{cases} 1 & \texttt{if garden=}\ 1\ (\texttt{Northern}) \\ 0 & \texttt{if garden=}\ 2\ (\texttt{Southern}) \end{cases}$$

ORDINAL COVARIATE : `time points (as Day number)` t_{ij} = `Day` j `for flower ID` i.

OUTCOME : Response variable

$$Y_{ij} = \begin{cases} 1 & \texttt{for a fresh outcome} \\ 0 & \texttt{otherwise} \end{cases}$$

PROBABILITY OF OUTCOME : π_{ij}, the probability that i^{th} flower is fresh at day point j.

There were 33 parameters to be estimated if we consider distilled water to be the reference to which the compounds are compared; *Hybrid Tea* species type (=2) the reference to which the *Floribunda* species type (=1) is compared; and Southern garden (=2) the reference to which the Northern garden is compared. The model was built to be able to account for the effect of `Compounds` separately, for the effect of `Species`, for the effect of `Garden`, the effect of `time` (ordered day points) and the effect of `time` × `compound` interactions.

7.2.2 *GEE with first-order autoregressive working correlation structure*

The equation (10) is ignoring the correlation structure due to the repeated measurements within flowers over time. This would be correct if measurements at different time points would also be taken on different flowers. Actually, the results obtained from this equation were used as starting values in the fitting of a more realistic model that do account for the association structure. We adopted as association structure a working correlation matrix that is autoregressive to the first order AR(1).

Two outcomes of the very same flower are correlated but the strength of the correlation decreases if the two outcomes are farther apart in time. The longer the number of days between the two outcomes, the smaller the correlation between the two outcomes for the same flower. More specifically, the working correlation structure that was assumed and was used for fitting and estimation purposes is given by (first-order autoregressive correlation structure):

$$\text{Corr}\,(Y_{ij}, Y_{ij+\tau}) = \alpha^{\tau} \quad (11)$$

$$\tau \in [0, \ldots, 30 - j] \quad (12)$$

$$j \in [0, \ldots, 30] \quad (13)$$

8 RESULTS AND STATISTICAL ANALYSIS

8.1 Exploratory data analysis

8.1.1 *Missing values*

While the experiment proceeded, 26 flowers were damaged, out of which 4 had already failed the freshness criterion. As a result, there were 22 missing data values. The 22 *true* missing values have been deleted from the dataset. From the random nature of the event that caused the missingness, the 22 true missing values were considered as missing at random values. The final dataset used for the statistical analysis had 2678 observations (2678 flowers ID). The missing values accounted for less than 0.815% of the total number of observations and did not impair the targeted power of the experiment.

8.1.2 *Graphical exploration*

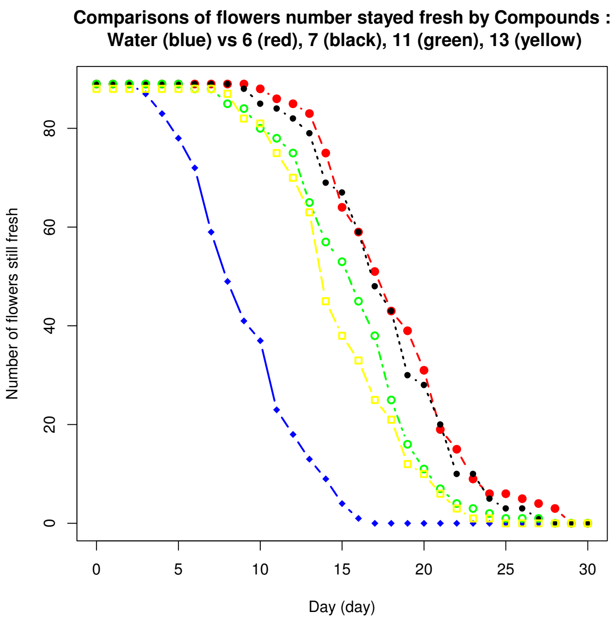

Figure 5: Number of flowers stayed fresh at a given Day, *Floribunda* sp.

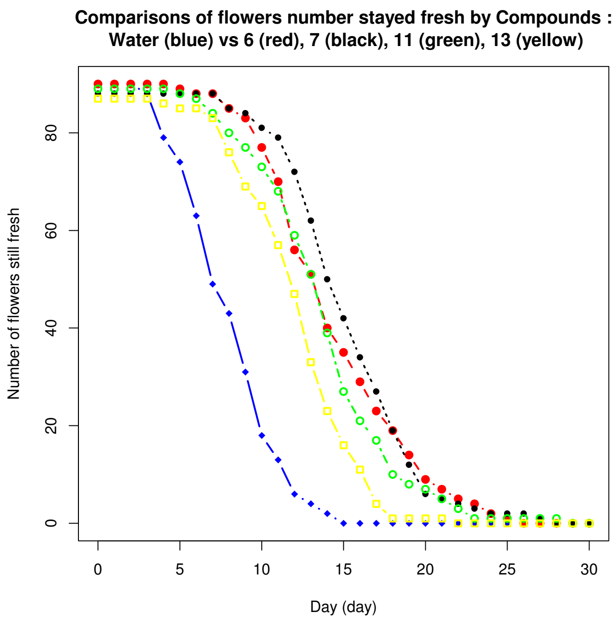

Figure 6: Number of flowers stayed fresh at a given Day, *Hybrid Tea* sp.

Figures 5 and 6 show the time evolution of the observed number of flowers stayed fresh at a given day for both species averaged by garden, for a selection of the best compounds compared to water (blue). Four main observations were made from the data exploration.

1. The time evolution pattern of the number of days flowers stayed fresh depended on the compound.
2. Compound 7 seemed to have the longest average number of fresh days (15.8 days).
3. The average number of days was higher for *Floribunda* sp. than for *Hybrid Tea* sp.
4. The compound ranking (best to worst) remained the same whatever the species and whatever the garden.

8.2 Poisson regression analysis and results

The Poisson regression model (equation 6) with the working correlation assumption with respect to the blocks expressed by equation 7 were fitted to the data (see SAS code #2 and code #3 in the appendix).

8.2.1 *Poisson regression parameter estimates (standard version)*

The maximum likelihood parameter estimates are given on table 3 in the standard version (no underlying correlation structure, i.e. no GEE). We noted immediately that there is no *between* block effect ($H_0 : \beta_{20} = 0$

Table 3: Maximum Likelihood Parameter Estimates of the standard Poisson regression.

Name	Parameter	Estimate	Standard Error	Wald 95% CL		Wald χ^2	Pr>χ^2
Int.	β_0	1.9714	0.0309	1.91	2.03	4081.4	< .0001
Comp.2	β_2	-0.3355	0.0414	-0.42	-0.25	65.78	< .0001
Comp.3	β_3	0.3037	0.0352	0.23	0.37	74.60	< .0001
Comp.4	β_4	0.2151	0.0358	0.14	0.28	36.02	< .0001
Comp.5	β_5	0.4006	0.0345	0.33	0.47	134.74	< .0001
Comp.6	β_6	0.6946	0.0327	0.63	0.76	451.13	< .0001
Comp.7	β_7	0.7070	0.0327	0.64	0.77	467.78	< .0001
Comp.8	β_8	0.0299	0.0375	-0.04	0.10	0.63	0.4258
Comp.9	β_9	0.2122	0.0359	0.14	0.28	34.90	< .0001
Comp.10	β_{10}	-0.6771	0.0460	-0.77	-0.59	216.68	< .0001
Comp.11	β_{11}	0.5911	0.0333	0.53	0.66	314.64	< .0001
Comp.12	β_{12}	0.2050	0.0360	0.13	0.28	32.48	< .0001
Comp.13	β_{13}	0.5057	0.0340	0.44	0.57	221.76	< .0001
Comp.14	β_{14}	-0.5558	0.0443	-0.64	-0.47	157.25	< .0001
Comp.15	β_{15}	0.2237	0.0358	0.15	0.29	38.98	< .0001
Comp.1 (water)	β_1	0.0000	0.0000	0.00	0.00	.	.
Floribunda	β_{16}	0.2309	0.0124	0.21	0.26	347.91	< .0001
Hybrid Tea	β_{17}	0.0000	0.0000	0.00	0.00	.	.
Northern	β_{18}	-0.0622	0.0122	-0.09	-0.04	25.86	< .0001
Southern	β_{19}	0.0000	0.0000	0.00	0.00	.	.
Block	β_{20}	-0.0008	0.0022	-0.005	0.0035	0.12	0.7281

is not rejected, p-value=0.73). Only one compound (compound 8) was not significantly different from water. Three compounds (compound 2, 10, 14) had a significantly more negative effect than water. Ten compounds had a significantly more positive effect than water. Two compounds (compound 6 and 7) were performing almost equally well and were qualified as the best compounds with respect to the number of days flowers stay fresh. Exponentiating the β regression coefficient for the compounds results in the multiplicative effect (incident rate ratio) on the number of days a flower stays fresh with respect to the reference (water). Hence, for compound 7, $e^{0.707} = 2.03$: the expected number of days a flower stayed fresh is more than twice as much longer than when compared to water. The incident rate ratio is $e^{0.2309} = 1.26$ when moving from *Hybrid Tea* sp. to *Floribunda* sp. The *Floribunda* sp., on average, lives 26% longer than *Hybrid Tea* sp. in terms of number of days a flower stays fresh. The incident rate ratio for the garden effect is $e^{-0.0622} = 0.94$: flowers grown in the northern garden stay fresh, on average, a shorter number of days than the southern garden grown flowers (6% shorter).

From the regression parameter estimates, it appeared that compound 6 and 7 were the best compounds as compared to water with respect to the research question. A formal contrast test was carried out to test for a significant difference between compound 6 and compound 7. The results of the contrast statement in the SAS code were the following :

```
                                          Chi-
Contrast                         DF     Square   Pr > ChiSq   Type
Compound 6 - 7 :                  1       0.22       0.6418   LR
Compound 8 - 1 (water) :          1       0.63       0.4258   LR
```

The result was that compounds 6 and 7 had not a significantly different effect on the number of days the flower stayed fresh (p-value=0.64) when compared to each other. Taken separately, each of compound 6 and 7 were significantly better than water. It was also noted that compound 8 was not significantly different than water.

8.2.2 *Flower lifetime marginal and conditional averages by Compound*

The expected numbers of days flowers stay fresh (expected flower lifetime) are given in table 4 together with the expected lifetime, conditional on the Garden and Species covariates levels, for each compound. The expected number of days that the flowers can stay fresh, depending on the type of compound, are displayed with their 95% confidence interval in figure 7, averaged for garden and species effects.

There is a species (= Type) effect. On average, other covariates held constant, the number of days *Floribunda* sp. flowers stay fresh is 1.26 times longer than *Hybrid Tea* sp. flowers stay fresh.
There is also a Garden effect. On average, other covariates held constant, the number of days the flowers grown in the Northern Garden stay fresh is 0.94 times the number of days they would have stayed fresh if grown in the Southern Garden.

Table 4: Expected Lifetime ($\bar{\lambda}$) and Expected conditional Lifetime ($\bar{\lambda}|(G, S, B)$) of flowers by compound (in days).

Compound	Garden	Southern Garden		Northern Garden	
	Species	*Floribunda* sp.	*Hybrid Tea* sp.	*Floribunda* sp.	*Hybrid Tea* sp.
	$\bar{\lambda}$	$\bar{\lambda}\|(G=2, S=2)$	$\bar{\lambda}\|(G=2, S=1)$	$\bar{\lambda}\|(G=1, S=2)$	$\bar{\lambda}\|(G=1, S=1)$
1	7.78 [7.38-8.20]	9.04	7.17	8.49	6.74
2	5.56	6.46	5.13	6.07	4.82
3	10.54	12.25	9.72	11.51	9.14
4	9.65	11.21	8.90	10.53	8.36
5	11.61	13.49	10.71	12.68	10.06
6	15.58 [15.02-16.17]	18.10	14.37	17.01	13.50
7	15.78 [15.20-16.37]	18.33	14.55	17.22	13.67
8	8.02	9.31	7.39	8.75	6.95
9	9.62	11.18	8.87	10.50	8.34
10	3.95	4.59	3.65	4.31	3.43
11	14.05	16.32	12.96	15.34	12.18
12	9.55	11.10	8.81	10.43	8.28
13	12.90	14.99	11.90	14.08	11.18
14	4.46	5.18	4.12	4.87	3.87
15	9.73	11.30	8.97	10.62	8.43

8.2.3 *Poisson Regression Goodness of Fit Diagnostics*

As described by Molenberghs and Verbeke (2005) [7](p.206), the goodness of fit evaluation with the deviance D^2 and Pearson χ^2 is correct only in the context of cross-sectional data. Applying the method to analyse repeated measures should ignore the information on goodness of fit. Hence, this information will be ignored with the logistic regression model where we have repeated measures over time. Here, with the Poisson regression and with the standard (non GEE approach), where the day counts are viewed as global independent endpoint measures for each flower, we can comment on deviance D^2 and Pearson χ^2 criteria assessing the goodness of fit. The deviance $D^2/\mathrm{df} = 1.19$ and Pearson $\chi^2/\mathrm{df} = 1.18$ were close to one and the Poisson regression fit appeared to be satisfying.

8.2.4 *GEE Estimates under Exchangeable Working Correlation Assumption*

In subsection 8.2.1, the parameter estimates obtained from fitting the model with the maximum likelihood algorithm, ignored the correlation structure within blocks. In other words, a standard Poisson regression was fitted, corresponding to an independent working assumption. In the present section, this independence assumption is challenged. The exchangeable working correlation assumption is adopted to conduct a GEE approach in the parameters estimation (a quasi likelihood analysis underlies the GEE approach). The SAS code # 3 in appendix identify `block` as the cluster variable, i.e., the variable that defines groups of related observations. The blocks are nested in the gardens. Indeed, the blocks are different clusters in the 2 gardens. In the initial analysis, the sample size was 2678. Now this is refined from a correlated data (GEE) setting : there are 20 clusters (10 blocks in each garden) with a cluster size of 135 flowers per cluster or 132 (due to deleted missing values). Two relevant subsets of parameter estimates are tabulated : both the empirical and model based standard errors estimates are given in table 5. The sets of parameters are identical but the empirically corrected standard errors are a bit larger than the model based ones. The empirical standard error estimates and the model-based standard error estimates are rather close to each other and most importantly they are the same whether we use the independent working correlation assumption or the exchangeable working correction assumption. The p-values (not tabulated here) lead to the same

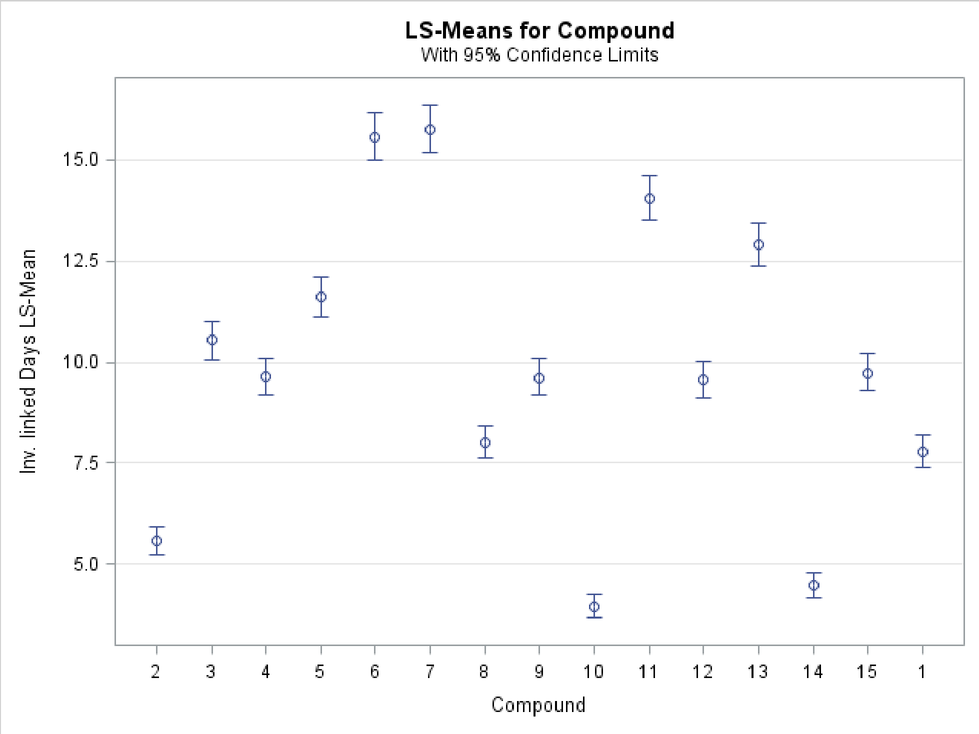

Figure 7: Expected number of Days flowers stay fresh per compound, averaged for garden and species effects.

conclusion as before. The asymptotic variance-covariance matrices differ. The result for ρ is -0.000273552, the estimated exchangeable working correlation , in our equation 7. These results informally indicate that the exchangeable correlation is extremely moderate (if any) and the working correlation matrix seems to exhibit no correlation between pairs of flowers within a block. We concluded that the split plot randomized block, as expected, had properly balanced hidden edge effects *within* blocks (and *between* blocks as well).

The block covariate could now be removed from our subsequent analysis because there was no block effect and we could rely on the independence assumption *within* and *between* blocks as well. A correlation structure within the block was not incorporated anymore in the subsequent analysis.

Table 5: Poisson regression GEE estimates under exchangeable working correlation within blocks.

Effect	Par.	Standard~IND		GEE under EXCH	
		estimate	(empir. ; model)	estimate	(empir. ; model)
Int.	β_0	1.9624	(0.0381 ; 0.0366)	1.9623	(0.0381 ; 0.0363)
...	...	...	...	...	...
Comp.6	β_6	0.6945	(0.0479 ; 0.0355)	0.6945	(0.0479 ; 0.0355)
Comp.7	β_7	0.7070	(0.0435 ; 0.0355)	0.7070	(0.0435 ; 0.0355)
...	...	...	...	...	...
Floribunda	β_{16}	0.2554	(0.0106 ; 0.0299)	0.2557	(0.0106 ; 0.0294)
Northern	β_{18}	-0.0623	(0.0090 ; 0.0133)	-0.0624	(0.0090 ; 0.0131)

8.3 Logistic regression analysis and results

8.3.1 *Data manipulation step for logistic regression*

In order to conduct the logistic regression described by equation (10) and with the working correlation structure described by equation (12) as generalized estimating equation (GEE), the dataset had to be manipulated so that the single line repeated day points measurements per ID were converted in multiple lines per ID (one observation line per day point). The SAS code for this data step is the first part of code #4 in the appendix. The REPEATED statement identifies the `ID` of the flowers as the SUBJECT of the repeated measurements. The variable `DAY_POINT_class` identifies the discrete time points, expressed as numbered days, over which the same IDs are repeatedly measured. The TYPE=AR option specifies the working correlation assumption expressed by equation 12 . We noted that the algorithm converged.

8.3.2 *Logistic regression Parameter Estimates*

The magnitude of the Compound, Species (=Type), Garden effects, time effect and Compound ∗ time effect are assessed using the Type 3 likelihood score test.

```
 Score Statistics For Type 3 GEE Analysis
                                              Chi-
    Source                    DF            Square    Pr > ChiSq
    Compound                  14            205.77        <.0001
    Type                       1            295.58        <.0001
    Garden                     1             24.69        <.0001
    DAY_POINT                  1           2638.07        <.0001
    DAY_POINT*Compound        14            151.23        <.0001
```

All the covariates appeared to be significant.

The Compound ∗ time interaction was significant. This indicated that the patterns of change in the odds of a fresh outcome for the flowers over time were not the same for the different compounds. This was the most important result. The best compounds were the ones for which the combination of the compound term and the interaction term were the highest (and positive).

A few relevant GEE parameter estimates with their empirical and model based standard errors are tabulated in table 6. With the logistic regression model, the obtained parameter estimates can be interpreted in terms of odds. To compute the odds of observing a fresh outcome for a flower at day 15, for instance, we begin by computing the log-odds from the fitted GEE model (see table 6) :

$$\text{Water (reference) at day 15} \quad : \quad 4.2031 + 0 - 0.5615 \times 15 + 0 = -4.2194$$
$$\text{Compound 6 at day 15} \quad : \quad 4.2031 + 1.8339 - 0.5615 \times 15 + 0.1577 \times 15 = -0.02$$

Exponentiating the obtained log-odds, we obtained the odds and could compute the expected probabilities for the flowers to have a fresh outcome at day 15. For water at day 15, the odds are $e^{-4.2194} = 0.0147$. For compound 6 at day 15, the odds are $e^{-0.02} = 0.98$. The probability (π) is easily calculated from the odds :

$$\text{odds} = \frac{\pi}{1-\pi} \tag{14}$$

For water, at day 15, we have $\pi = 0.014$ and for compound 6 at day 15, we have $\pi = 0.495$. Since the references are *Hybrid Tea* sp. and the Southern Garden, the results correspond to those reference species and reference garden.

Table 6: Logistic regression GEE estimates under autoregressive working correlation assumption for fresh outcome of flowers repeatedly measured over day time points.

Parameter		Estimate	Std. error (empir. ; mod.b.)	95% CL		Z	Pr > |Z|
Intercept	β_0	4.2031	(0.2544 ; 0.3123)	3.7045	4.7018	16.52	< .001
...	...	...	...	...	...	...	...
Comp.6	β_6	1.8339	(0.4143 ; 0.4963)	1.022	2.6458	4.43	< .001
Comp.7	β_7	2.7591	(0.4767 ; 0.5473)	1.8249	3.6933	5.79	< .001
...	...	...	...	...	...	...	...
Comp.1 (water)	β_1	0.0000	(0.0000 ; 0.0000)	0.0000	0.0000	.	.
Floribunda	β_{16}	1.2563	(0.0719 ; 0.0691)	1.1155	1.3972	17.48	< .001
Hybrid Tea		0.0000	(0.0000 ; 0.0000)	0.0000	0.0000	.	.
Northern	β_{17}	-0.3485	(0.069 ; 0.0663)	-0.4838	-0.2132	-5.05	< .001
Southern		0.0000	(0.0000 ; 0.0000)	0.0000	0.0000	.	.
Day Point	β_{18}	-0.5615	(0.0276 ; 0.0340)	-0.6156	-0.5075	-20.37	<.0001
...	...	...	...	...	...	...	...
Day Point*Comp.6	β_{24}	0.1577	(0.0345 ; 0.0410)	0.0902	0.2253	4.58	<.0001
Day Point*Comp.7	β_{25}	0.1080	(0.0366 ; 0.0431)	0.0363	0.1798	2.95	0.0032
...	...	...	...	...	...	...	...
Day Point*Comp.1	β_{33}	0.0000	(0.0000 ; 0.0000)	0.0000	0.0000	.	.

The Species effect and Garden effect can be corrected with the corresponding parameter estimates (β_{16} and β_{17}).

There is a significant Species effect :the odds of a fresh outcome for Floribunda are $e^{1.2563} = 3.51$ times the odds for Hybrid Tea on average. There is a significant garden effect : the odds of a fresh outcome with flowers from the Northern garden are $e^{-0.3485} = 0.68$ times the odds of flowers from the Southern garden on average.

More complete predicted probability estimates are provided in table 7 below.

8.3.3 *Working correlation assumption assessed*

The autoregressive working correlation matrix was estimated and gave the value for $\alpha = 0.7518$. It is a 30×30 dimensional matrix (30 days of repeated measures on each flower) with ones on the diagonal and 0.7518 in the first off-diagonal band and then each next band is again multiplied by 0.7518. The further away from the diagonal, the smaller the correlation. This estimated correlation of 0.7518 was rather high, indicated that two outcomes 1 day apart were highly correlated, whereas two outcomes 10 days apart had a weaker correlation $0.7518^{10} = 0.0577$.

We compared the empirical and model based standard errors of the logistic regression parameter estimates under the AR working correlation with other working correlation assumptions (exchangeable and independence). The empirical and model based standard errors are much closer to each other when the auto-regressive working correlation assumption is used. This result showed that our choice was reasonable and proved appropriate in providing good efficiency in the parameters estimation under the autoregressive working correlation assumption.

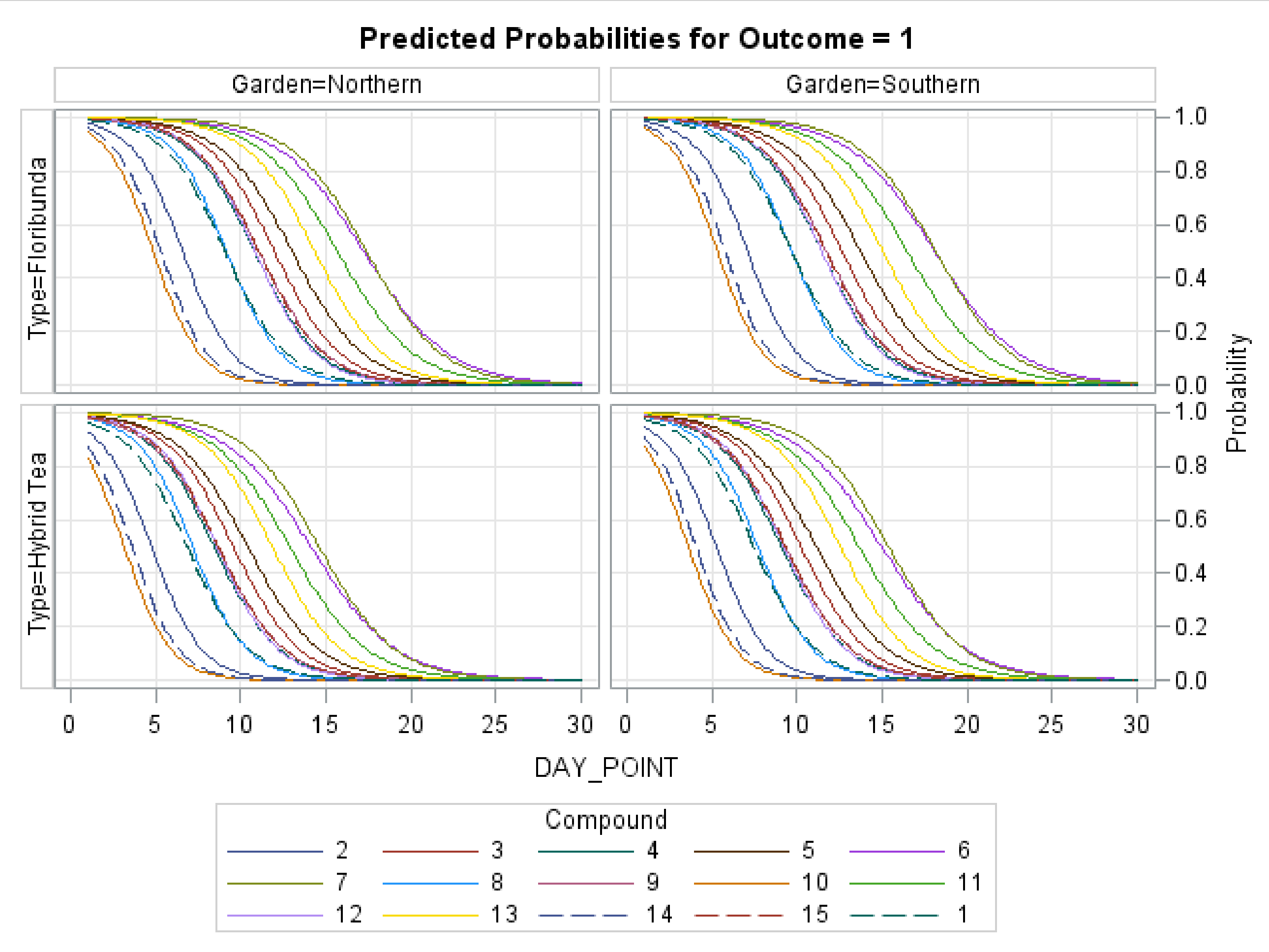

Figure 8: Predicted probability that a flower outcome is fresh over days point per species, per garden and per compound.

8.3.4 *Outcome Probability per day species and garden for the selected best 2 compounds as compared to water*

The predicted probabilities of observing a flower being fresh at a given day conditional on Species and Garden are graphically displayed on figure 8. Comparing rows and columns on this figure show the Species effect and the garden effect together with the Compound effect. Table 7 displays predicted probabilities of a fresh outcome for flowers, conditional on Species and Garden for the two best compounds and are compared to water.

Table 7: Predicted probability for a fresh status outcome of a flower per day point, for the 2 best compounds, per garden and species, as compared to water.

Day(j)	SOUTHERN GARDEN WATER (Comp.1) $E(\pi_j)$ Flori-bunda	SOUTHERN GARDEN WATER (Comp.1) $E(\pi_j)$ Hybrid Tea	SOUTHERN GARDEN Compound 6 $E(\pi_j)$ Flori-bunda	SOUTHERN GARDEN Compound 6 $E(\pi_j)$ Hybrid Tea	SOUTHERN GARDEN Compound 7 $E(\pi_j)$ Flori-bunda	SOUTHERN GARDEN Compound 7 $E(\pi_j)$ Hybrid Tea	NORTHERN GARDEN Compound 6 $E(\pi_j)$ Flori-bunda	NORTHERN GARDEN Compound 6 $E(\pi_j)$ Hybrid Tea	NORTHERN GARDEN Compound 7 $E(\pi_j)$ Flori-bunda	NORTHERN GARDEN Compound 7 $E(\pi_j)$ Hybrid Tea
8	0.725	0.428	0.983	0.943	0.990	0.966	0.976	0.921	0.991	0.952
10	0.461	0.196	0.963	0.948	0.975	0.919	0.946	0.839	0.966	0.889
15	0.049	0.014	0.755	0.495	0.805	0.540	0.708	0.409	0.744	0.453
16	0.029	0.008	0.697	0.395	0.724	0.427	0.619	0.316	0.649	0.345
17	0.017	0.005	0.605	0.304	0.625	0.321	0.520	0.236	0.540	0.250
18	0.009	0.003	0.506	0.226	0.514	0.231	0.333	0.171	0.427	0.175
19	0.005	0.002	0.406	0.163	0.402	0.160	0.326	0.121	0.322	0.119
20	0.003	0.001	0.314	0.115	0.299	0.108	0.244	0.084	0.231	0.079

9 DISCUSSIONS AND CONCLUSIONS

The study provided evidence of a significant effect of compounds in the patterns of change in the flower freshness outcomes over day time points.

The study successfully determined that two compounds had the best positive effect on the number of days a cut flower could stay fresh. An effect size of 10% was requested in the experimental design as the threshold measure of success of the study, corresponding to a multiplicative factor of 1.10 (Incident Rate Ratio) in the number of days a flower could stay fresh when compared to a treatment with water alone. Compounds 6 and compounds 7 surpassed this success threshold significantly with an Incident Rate Ratio of 2, corresponding to a doubling in the average number of days the flowers stayed fresh on average, when compared to water alone. The two best compounds 6 and 7 show similar results and are not significantly different from each other. Any of these two compounds (6 or 7) can safely be recommended with respect to the addressed research question.

The study has also shown that the ranking of the compounds from best to worse is the same whatever the species and whatever the garden. It is a favorable indication that a compound which showed better results than another one for a particular species will probably also be better than the other one for another species or in another garden (there were no evidence of compound $*$ species interaction nor compound $*$ garden interactions).

However, the study has also shown that there is a significant species effect and a significant garden effect. The precise results of the predicted odds are thus species dependent and garden dependent. On average, the odds ratio for a freshness outcome for *Floribunda* sp. compared to *Hybrid Tea* is 3.51. On average, the odds ratio for a freshness outcome for the northern garden compared to the southern garden is 0.706.

As a quantitative useful illustration of the study results, it is indicated that the probability for a flower to still be fresh at day 20, when treated and conditioned with compound 6, is 0.31 if the flower is a *Floribunda* specimen. This probability when we move from *Floribunda* to *Hybrid Tea* specimens decreases to 0.11. These results are predicted for flowers that were grown in the southern garden. The odds for being still fresh after 20 days are smaller for flowers grown in the northern garden. The predicted probability to still be fresh at day 20 decreases to 0.24 and 0.08 respectively for *Floribunda* sp. and *Hybrid Tea* sp. in the northern garden.

Liability has been disclaimed for a direct transposition of these conclusions to the Black Tulip. The study has shown strong evidence that probabilities of particular outcomes are species dependent but also location dependent.

10 APPENDIX

CODE #1 : Sample Size calculation with SAS

```
/*--------- Poisson regression for one sided approximation--------------------*/
title 'Power analysis for Poisson, 2 treatments';
data power_poisson;
input trt $ mean;
reps=178;
do obs=1 to reps;
output;
end;
datalines;
control 7.30
exper 8.03
;
run;
proc print noprint
data=power_poisson;
run;
proc glimmix data=power_poisson;
class trt;
model mean=trt/chisq link=log dist=poisson;
contrast 'control vs experimental' trt 1 -1 /chisq;
ods output tests3=F_overall contrasts=F_contrasts;
run;
data power;
set F_overall F_contrasts;
nc_parm=numdf*Fvalue;
alpha=0.10;
/* alpha is doubled for complying to the one sided tail */
/* this is the 0.05 version of the one sided sample size*/
F_crit=Cinv(1-alpha,numdf,0);
Power=1-probchi(F_crit,numdf,nc_parm);
proc print data=power;
title3 'power for sample size = N=178 per Trt';
run;
```

CODE #2 : Poisson Regression with SAS (standard version).

```
ods graphics on;
proc GENMOD data=rep.TulipNOMISS;
class Garden Type Compound(ref=FIRST);
model Days=Compound Type Garden Block/dist=poisson link=log type3;
lsmeans Type Garden Compound /ilink cl plots=meanplot(cl ilink);
contrast 'Compound 6 - 7 :' Compound 0 0 0 0 1 -1 0 0 0 0 0 0 0 0 0;
contrast 'Compound 6 - 1 (water) :' Compound 0 0 0 0 1 0 0 0 0 0 0 0 0 0 -1;
contrast 'Compound 7 - 1 (water) :' Compound 0 0 0 0 0 1 0 0 0 0 0 0 0 0 -1;
contrast 'Compound 8 - 1 (water) :' Compound 0 0 0 0 0 0 1 0 0 0 0 0 0 0 -1;
store Poisson_Stored;
ods output LSMeans=LogLambda;
run;
ods graphics off;
```

CODE #3 : Poisson Regression with SAS (GEE version under exchangeable working correlation assumption).

```
/* POISSON REGRESSION WITH GEE (BLOCK REPEATED MEASURES of different*/
/* flowers within a BLOCK)                                          */
/* BLOCKS ARE NESTED IN GARDEN                                      */
proc GENMOD data=rep.TulipNOMISS;
class Block Garden Type Compound(ref=FIRST) ID;
model Days=Compound Type Garden Block/dist=poisson link=log;
repeated subject= Block(Garden) /withinsubject=ID type=exch covb modelse;
run;
```

CODE #4 : Logistic Regression with SAS (GEE version under autoregressive working correlation assumption).

```
/* Restructuring the file : creating multiple ID lines from a single    */
/* line with 30 days of observations (using an array)                   */
/* The new restructured dataset will be 2678 x 30 = 80340 lines         */
data rep.Tulip4LOGISTIC;
set rep.TULIPNOMISS;
array FDay[30];
/* the purpose of the previous line is to assign FDay[30]=FDay1-FDay30  */
/* All the flowers are fresh on FDay0 !                                 */
do DAY_POINT = 1 to 30;
Outcome=FDay[DAY_POINT];
DAY_POINT_class=DAY_POINT;
output;
end;
keep ID Days Compound Type Garden Block Cell DAY_POINT DAY_POINT_class Outcome;
run;
```

```
ods graphics on;
proc GENMOD data=rep.Tulip4LOGISTIC descending;
class ID Compound(ref=FIRST) Type Garden DAY_POINT_class;
model Outcome=Compound Type Garden DAY_POINT Compound*DAY_POINT/dist=binomial link=logit type3;
repeated subject=ID /withinsubject=DAY_POINT_class type=AR covb corrw modelse;
effectplot slicefit(sliceby=Compound plotby(rows)=Garden) /at(Type='Floribunda' 'Hybrid Tea') noobs;
store logiresults;
run;
ods graphics off;
```

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