Plants stand still but hide: imperfect and heterogeneous detection is the rule when counting plants

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Abstract

1. The estimation of population size and its variation largely relies on counts of individuals, generally carried out within spatial units such as quadrats or sites. Missing individuals during counting (i.e. imperfect detection) results in biased estimates of population size and trends. Imperfect detection has been shown to be the rule in animal studies, and most studies now correct for this bias by estimating detection probability. Yet this correction remains exceptional in plant studies, suggesting that most plant ecologists implicitly assume that all individuals are always detected.
2. To assess if this assumption is valid, we conducted a field experiment to estimate individual detection probability in plant counts conducted in 1x1 m quadrats. We selected 30 herbaceous plant species along a gradient of conspicuousness at 24 sites along a gradient of habitat closure, and asked groups of observers to count individuals in 10 quadrats using three counting methods requiring progressively increasing times to complete (quick count, unlimited count and cell count). In total, 158 participants took part in the experiment, allowing an analysis of the results of 5,024 counts.
3. Over all field sessions, no observer succeeded in detecting all the individuals in the 10 quadrats. The mean detection rate was 0.44 (ranging from 0.11 to 0.82) for the quick count, 0.59 for the unlimited count (range 0.18–0.87) and 0.74 for the cell count (range 0.46–0.94).

4. Detection probability increased with the conspicuousness of the target species and decreased with the density of individuals and habitat closure. The observer's experience in botany had little effect on detection probability, whereas detection was strongly affected by the time observers spent counting. Yet although the more time-consuming methods increased detection probability, none achieved perfect detection, nor did they reduce the effect on detection probability of the variables we measured.
5. *Synthesis and applications.* Given that plant detection was always imperfect and highly heterogeneous, rather than rely on raw counts, plant ecologists should use methods that estimate the detection probability of individuals to assess the size, temporal or spatial trends of plant populations.

Keywords: conservation; demography; detectability; field methods; N-mixture; observer effect; plant survey; population monitoring.

Introduction

The size of populations and their variation over time and space are of central importance in ecology and conservation. These parameters are required to understand ecosystem dynamics (Ripple & Beschta, 2012; Watson & Estes, 2011), assess population viability (Dennis et al., 1991) and trigger conservation policies and actions (e.g. IUCN, 2019). Their estimation largely relies on counts of individuals, generally made in spatial units such as quadrats or sites (Elphick, 2008; Yoccoz et al., 2001). Therefore, the accuracy of these counts is crucial, as counting errors would result in biased estimates of population sizes and trends.

When carrying out counts, observers can miss some individuals, in effect counting fewer individuals than were present in the sampling units. This phenomenon of imperfect detection, well known in animal studies, leads to underestimating population sizes (Williams et al., 2002). If detection is imperfect but constant over time and space, raw counts can provide unbiased estimates of population trends or spatial variation (Yoccoz et al., 2001). However, if detection varies over time (e.g. across the sampling season or between years) or space (e.g. between habitats), the raw differences between counts performed at different sampling sessions or in different sampling units are a mixture of differences in abundance and detection (Kéry et al., 2009). For example, a decrease in population size over the years may be hidden as observers improve their ability to detect individuals over time (Kendall et al., 1996). Or one might conclude that there are fewer individuals in closed than in open habitats because they are harder to detect in closed habitats (Buckland et al., 2008). Even differences as small as 4–8% in detection dramatically increase the risk of erroneously concluding that population size varies over time or space (Archaux et al., 2012).

Since the 1970s, many studies on vertebrates have shown that imperfect detection is the rule and that detection probability tends to vary in time and space. Detection probability depends on a number of factors: the observation process (both the observation method and the survey effort), the visibility of the target species, and the characteristics of its habitat (Kéry et al., 2005; Kéry & Schmidt, 2008; Petitot et al., 2014). Consequently, studies including an estimate of detection probability, which corrects for the bias resulting from imperfect detection, have become increasingly common for vertebrates and, to a lesser extent, invertebrates. Yet they remain exceptional for plants (Kellner & Swihart, 2014), suggesting that most plant ecologists implicitly assume that all individuals are always detected, or that differences in detection probability are negligible. It is true that, unlike most animals, plants are immobile, and observers may thus consider it unlikely to miss individuals if careful sampling is conducted. This assumption led John Harper to make the famous statement "Plants stand still and wait to be counted," comparing the ease of studying plant and animal populations (Harper, 1977).

Although detection has almost never been investigated in plants at the individual level, some studies have investigated it at the species level, assessing species occurrence in a given area using two main approaches: occupancy or time-to-detection designs. In the first approach, the survey duration is fixed, and the detection probability of the species when surveying quadrats or sites is estimated from repeated surveys. In the second approach, detection probability is estimated based on the survey time required to detect the species. These studies found that the detection of plant species was almost always imperfect, and that detection probability varied depending on ecological and observational variables. For instance, species detection varied according to the morphology of the target species (Chen et al., 2009, 2013; Garrard et al., 2013), with conspicuous growth forms and phenological stages raising detection

probability. A high density of individuals increased the probability of detecting the species (Bornand et al., 2014), while the closure of the surrounding habitat decreased it (Alexander et al., 2012; Garrard et al., 2008). Species detection probability also increased with survey effort (Chen et al., 2009) and often varied between observers (Garrard et al., 2008; Moore et al., 2011). While these studies confirm that detection issues exist when assessing plant species occurrence, do these issues affect individual detection to a similar extent?

Concerning detection at the individual level, the only studies that have been conducted on plants used marked individuals in so-called 'capture–recapture' designs (Lebreton et al., 1992) or the time-to-detection of individuals (Hauser et al., 2022). These studies found that individual detection was imperfect, although individuals were generally searched for in relatively small, intensively surveyed plots (Alexander et al., 1997; Andrieu et al., 2017; Kéry & Gregg, 2003). In most studies using marked individuals, individual detection probability varied with the phenological stage of individuals and sometimes with habitat closure (Andrieu et al., 2017). Hauser et al. (2022) showed that flowering individuals were detected faster than non-flowering ones, and that the abundance of individuals of species resembling the target species delayed detection. Observers with recent experience in searching for the target species detected it faster, confirming at the individual level what has been demonstrated at the species level. Therefore, it is particularly concerning that individual detection probability has never been estimated for counts of unmarked plants, despite this being one of the most commonly used methods for estimating population sizes and trends in plant ecology (Reisch et al., 2018).

To assess if imperfect detection of individuals is indeed the rule in plant counts, we conducted a field experiment in which observers counted plants in 1x1 m quadrats. In total, the

experiment covered 30 species, 300 quadrats and involved 158 observers who made 5,024 counts. The questions we wanted to answer were: (1) Are all individuals detected in plant counts? (2) Is individual detection probability homogeneous in time and space? (3) Does increasing the survey effort result in higher detection probability, and could this allow perfect detection to be reached? To explore these questions, we tested whether detection varies according to ecological variables (species conspicuousness, true population density and surrounding habitat closure) and observational variables (counting method and experience of observers), which are likely to vary over time and space, as well as testing varying degrees of survey effort.

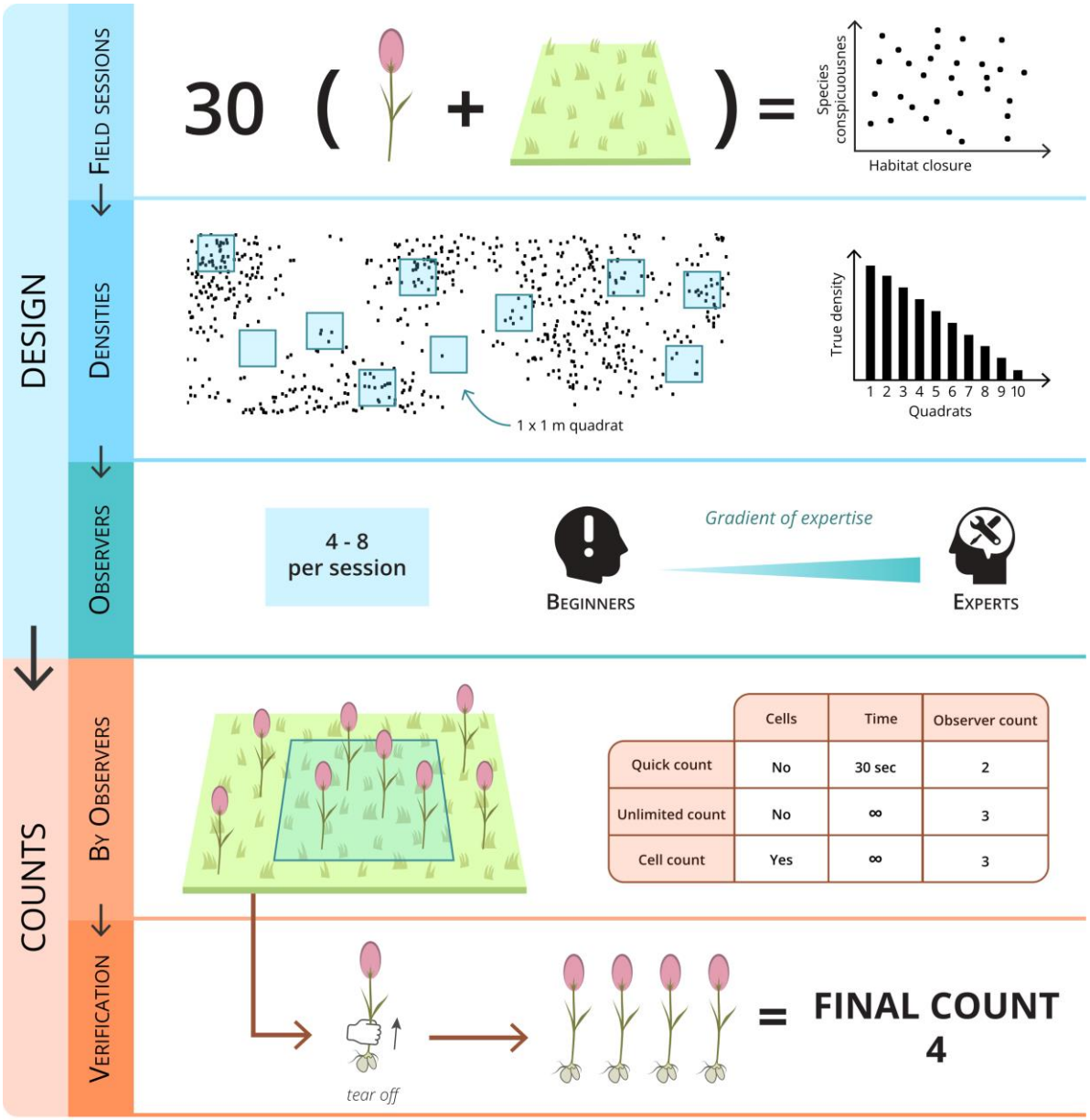
Materials & methods

1 – Selection of sites, species and observers

We selected 30 herbaceous plant species from 24 sites throughout France in Mediterranean, Alpine and temperate climatic regions (Appendix 1). We chose species with various growth forms and phenological stages to cover a gradient from cryptic to conspicuous species. As the experiment focused on counting individuals, we selected only species for which 'individuals' (ramets or genets depending on the species) could be unambiguously differentiated. We avoided species difficult to identify so that all could be identified at first glance without needing to check identification criteria on each detected individual. We selected the study sites to cover a gradient of habitat closure, from bare ground to dense meadows, to include all possible combinations of species conspicuousness and habitat closure (Fig. 1).

Participants were colleagues, researchers, undergraduate students and professionals involved in flora conservation. We selected 4 to 8 observers for each field session, including at least one with several years of field experience in botany, one with no experience in botany, and intermediate profiles. Observers were allowed to participate in up to three sessions, but these sessions had to be several weeks apart. This resulted in 171 participations by 158 unique observers. We conducted the fieldwork from 7 October 2020 to 6 August 2021.

2 – Experiment



	Cells	Time	Observer count
Quick count	No	30 sec	2
Unlimited count	No	∞	3
Cell count	Yes	∞	3

A

B

Figure 1: Diagram of the study's experimental design. We conducted 30 field sessions, each focusing on a different species in a habitat with a different closure level, to cover the widest possible range of combinations of species conspicuousness and habitat closure. For each field session, we placed ten quadrats on the population to form a density gradient and chose 4–8 observers to have a gradient of experience in botany. The observers carried out three counting methods successively (quick, unlimited and cell counts). We then counted the true number of individuals by systematically digging out the individuals detected by the observers and then conducting an additional survey for any individuals that remained unnoticed. Picture A shows the experiment in progress at a Mediterranean site, and picture B shows a quadrat with the cells.

147

148 Field set up and observer training

149 The fieldwork consisted of one-day sessions, during which a group of observers counted the
150 number of individuals of a target species in a habitat at one of the 24 sites. Before the
151 observers' arrival, we placed ten 1x1 m quadrats on the target species population. We chose
152 quadrat locations to ensure that (i) two were free of individuals of the target species (so that
153 observers would not always expect to find individuals in the quadrats), (ii) they covered a
154 relatively linear gradient of densities up to a maximum of 150 individuals (when this density
155 could be found on the site). We labelled the quadrats from 1 to 10 and fixed them to the
156 ground. When the observers arrived, we explained how the counts would be conducted and
157 how to identify the target species at all phenological stages and differentiate it from the other
158 species present at the study site. We then trained them to count on additional quadrats to
159 check that everyone had understood the instructions. This training phase lasted until all

160 observers felt comfortable with species identification and the counting methods. The whole
161 training process usually took about an hour.

162 Counting methods

163 Each observer had to count the number of individuals of the target plant in each quadrat using
164 three different methods, requiring a progressively increasing amount of survey time. In the
165 first round, participants had 30 seconds to estimate the number of individuals in each quadrat
166 (the quick count). In the second round, they had to count the number of individuals without
167 any time limit (the unlimited count). We then divided the quadrats into 100 cells of 10x10 cm
168 (Fig. 1B), and observers performed the third round, where they had to count the number of
169 individuals in each cell without any time limit (the cell count). This last method forced
170 observers to survey the quadrat homogeneously and has been regularly used in the plant
171 survey literature (e.g. Gauthier et al., 2017, 2019). For the unlimited and cell counts, observers
172 recorded the time they spent counting in each quadrat. To avoid memory effects, we collected
173 the sheets after each round, and observers surveyed the quadrats in a different order,
174 approaching it from a different side at each round. The observers conducted the three counts
175 on the ten quadrats in 1.5 to 5 hours (on average 3.25 hours).

176 Exhaustive count and measurement of covariates

177 After the observers had left, we carried out a final count to determine the true number of
178 individuals present in each quadrat. This was done by searching for all the individuals detected
179 per 10x10 cm cell by the observers during the third round and collecting them. Once all
180 individuals detected by at least one observer were removed, we searched again for any
181 potentially undetected individuals. This final count took between 1 and 7 hours (3 hours on

182 average), depending on habitat closure and the number of individuals of the target species in
183 the quadrats. We calculated the detection probability of individuals by dividing the number of
184 individuals detected by the observers by the true number of individuals present in each
185 quadrat.

186 We measured three variables to investigate their effect on detection probability: an index of
187 species conspicuousness (measured for each field session), an index of habitat closure
188 (measured for each quadrat) and the experience in botany of the observers. All the variables
189 and interactions we investigated in the following analysis are presented in Table 1, as well as
190 the underlying hypotheses. The way we measured the variables is detailed in Appendix 2.

191

192 3 – Statistical analysis

193 Overall, 300 quadrats were surveyed during the experiment by, on average, 5.7 observers,
194 resulting in 5,024 counts. We removed from the analysis 34 quadrats that contained no
195 individuals of the target species, as these do not provide information on detection probability.
196 We withdrew four additional quadrats that unexpectedly contained more than 300 individuals
197 to avoid the leverage effect due to these density outliers (Appendix 3). In some cases (N = 140,
198 representing 2.8% of the counts), the observers recorded more individuals than were truly
199 present. We thus carried out the analyses on two datasets, one in which we removed these
200 counts and one in which we set these counts to the true number of individuals. As there was
201 no discernible difference in the results, we present the results of the former analysis in the
202 main text and the latter in Appendix 7. After all the filtering steps, 4,459 counts remained in
203 the dataset from which we had removed the observations with excess detections. We also

204 performed the analyses keeping only the experienced botanists to check that our results were
205 not driven by an excess of novice observers in our sample. Despite some minor differences,
206 our overall results remained unchanged (Appendix 8).

207 We used generalised linear mixed models (GLMMs) with a binomial distribution and a logit
208 link function to analyse the correlation between detection probability and the variables we
209 measured. To take into account the repeated measurements at each quadrat, we included
210 random intercepts for the observers, the field sessions and the quadrats (nested within the
211 field session). We standardised all the variables prior to model fitting. We started by fitting a
212 model including all the variables and some of their interactions (see Table 1) and performed a
213 backward variable selection procedure, dropping every variable and interaction one by one,
214 keeping only the variables that decreased the Akaike information criterion (AIC) value of the
215 model (Burnham & Anderson, 2002). For the selected model, we computed the proportion of
216 variance explained by the model and the proportion of variance explained by the model's fixed
217 effects, using the theoretical variance method from Nakagawa et al. (2017). Analyses were
218 performed in R version 3.6.1 (R Core Team, 2019), using packages *lme4* (Bates et al., 2022) to
219 fit the models and *ggeffects* (Lüdecke et al., 2022) to plot the predictions.

220

221 Table 1: Ecological variables (species conspicuousness, habitat closure, true density),
222 observational variables (counting method, experience in botany), and interaction between
223 variables tested for their effect on the detection probability of individuals, with the hypothesis
224 of the underlying mechanism. The measurement method of all variables is detailed in
225 Appendix 2.

Variable	Description	Measurement method	Hypothesis	Type	Observed values
Species conspicuousness	Conspicuousness of the species at the time of the field session	Expert judgement based on the mean height of individuals and the presence of colourful flowers	Tall and colourful species are easier to detect, which increases their detection probability	Ordinal	1, 2, 3, 4
Habitat closure	Closure of the vegetation in the quadrat	Composite variable created from the vegetation height and the proportion of the area covered by vegetation in the quadrats (Appendix 2)	Dense vegetation masks individuals, which lowers detection probability	Continuous	-1.86–3.54
True density	Number of individuals present in the quadrat	Number of individuals counted in the quadrat by the experimenter at the end of the experiment	High density requires greater concentration and makes it difficult to distinguish between individuals, reducing detection probability	Count	0–295
Counting method	Counting method used	Fixed by the experimental design, each observer successively used the three counting methods on the ten quadrats	Counting methods requiring more survey time yield higher detection probability	Categorical	Quick count, unlimited count, cell count
Experience in botany	General experience in botany of the observer (not specific to the target species)	Self-evaluated by the observers before the experiment using a standardised form	Observers with more experience in botany have higher detection probability	Ordinal	1, 2, 3, 4, 5
Quadrat order + (Quadrat order)^2	Position of the quadrat in the round	Defined by the experimental design, the order in which the observers investigated the quadrats was fixed in advance and different for each observer and round. We added the squared variable to specify a quadratic relationship.	Learning increases detection probability over time, but fatigue decreases it	Continuous	1, 2, 3, …, 10

Counting time	Time spent counting by the observer	Each observer timed the duration of counting	Spending more time increases detection probability	Continuous	Quick count: 0.5 min Unlimited count: 0–11 min Cell count: 0–41.58 min
Species conspicuousness x Habitat closure			Detection probability is less affected by habitat closure for conspicuous species	Interaction term	
Species conspicuousness x Counting method			Detection probability obtained with counting methods requiring more survey effort is less affected by species conspicuousness	Interaction term	
Habitat closure x Counting method			Detection probability obtained with counting methods requiring more survey time is less affected by habitat closure	Interaction term	
Experience in botany x Counting method			The difference in detection probability between experts and beginners is larger for the quick count than for the other two methods	Interaction term	

True density x Counting method			Counting methods requiring more survey time make it easier to distinguish between individuals, which decreases the negative effect of true density	Interaction term	
(Quadrat order + (Quadrat order)^2) x Counting method			Counting methods requiring more survey time increase fatigue, which decreases detection probability	Interaction term	
Species conspicuousness x Counting time x Counting method		No coefficient is estimated for the quick count as the time was fixed	Spending an additional extra minute further increases detection probability for species that are easy to detect	Interaction term	
Habitat closure x Counting time x Counting method		No coefficient is estimated for the quick count as the time was fixed	Spending an additional extra minute further increases detection probability in sparse vegetation	Interaction term	

226

Results

Over the 30 field sessions, no observer succeeded in detecting all the individuals in all the quadrats. Averaged over all quadrats and observers, the mean detection rate per field session was 0.44 (ranging from 0.11 to 0.82) for the quick count, 0.59 for the unlimited count (from 0.18 to 0.87) and 0.74 for the cell count (from 0.46 to 0.94).

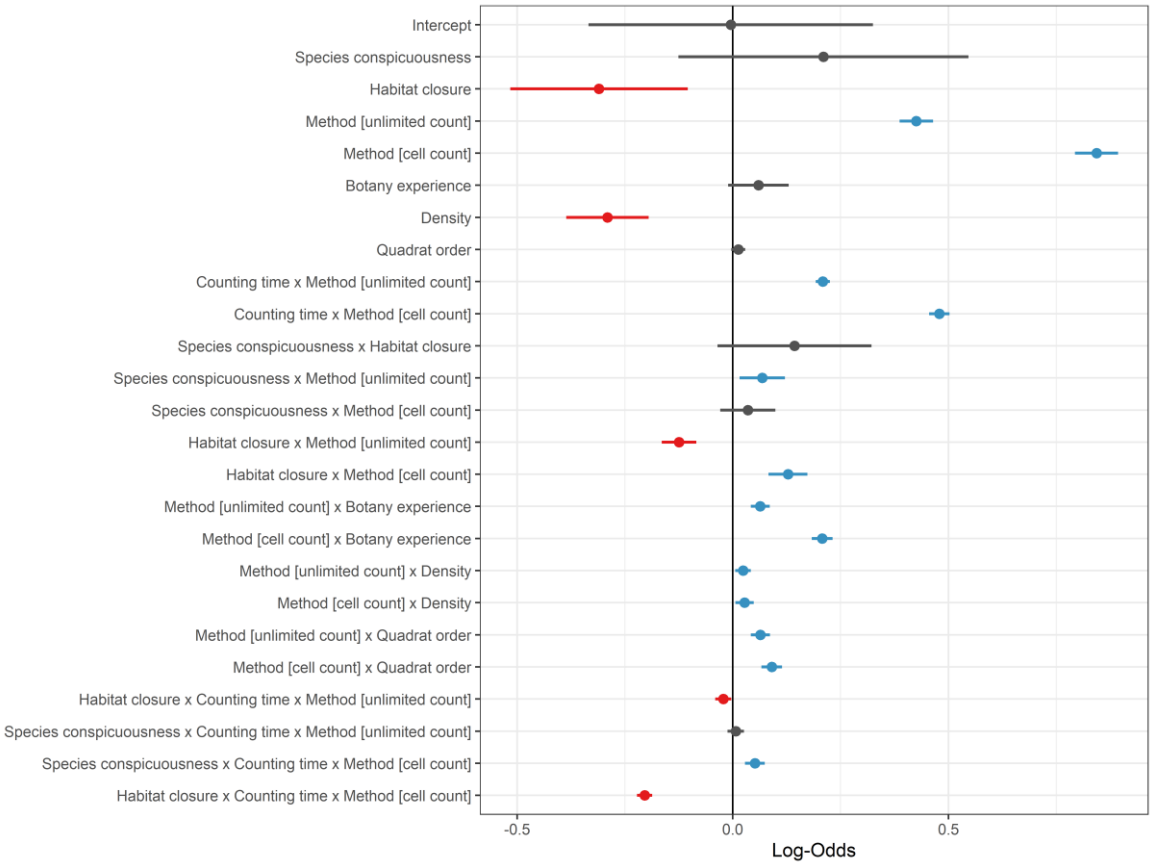


Figure 2: Coefficients of standardised variables in the best model. Dots represent estimated coefficients, and lines represent their 95% confidence intervals. Coefficients with a non-significant effect are shown in grey. The reference counting method is the quick count, in which the observers had 30 seconds to estimate the number of individuals in the quadrat. The coefficients presented for the two other methods indicate the difference (on the logit scale) in mean detection probability obtained with those methods compared to the quick count. The variable 'counting time' appears twice because we manually coded the interaction between the counting time and the counting method, so no slope parameter is estimated for the quick count.

After the backward selection procedure, the best model included all the tested variables and interactions except the squared quadrat order (Appendix 5). All ecological and observational variables were significantly correlated with detection probability in at least some conditions, i.e. for some counting methods or some levels of the other variables (Fig. 2; Appendix 6). Regarding the ecological variables, as expected, the more closed the habitat, the lower the detection probability (Fig. 3). The more conspicuous the species, the higher the detection probability, except in entirely open habitats, where the detection probability was constant and high regardless of the conspicuousness of the species (Fig. 3). Detection probability decreased steeply with true density, in a similar way for the three counting methods (Fig. 4). Regarding the observational variables, spending more time sharply increased detection probability for the unlimited and cell counts. Habitat closure moderated this trend, i.e. spending an extra minute counting increased detection probability less in closed habitats than in open habitats. Experienced observers achieved slightly higher detection probability than

246 inexperienced ones, and this difference was stronger for the cell count than for the unlimited
247 count. In contrast, there was no significant correlation between experience and detection
248 probability for the quick count. For the unlimited and cell counts, detection probability slightly
249 increased with the position of the quadrat in the round, while there was no correlation for the
250 quick count (Fig. 4).

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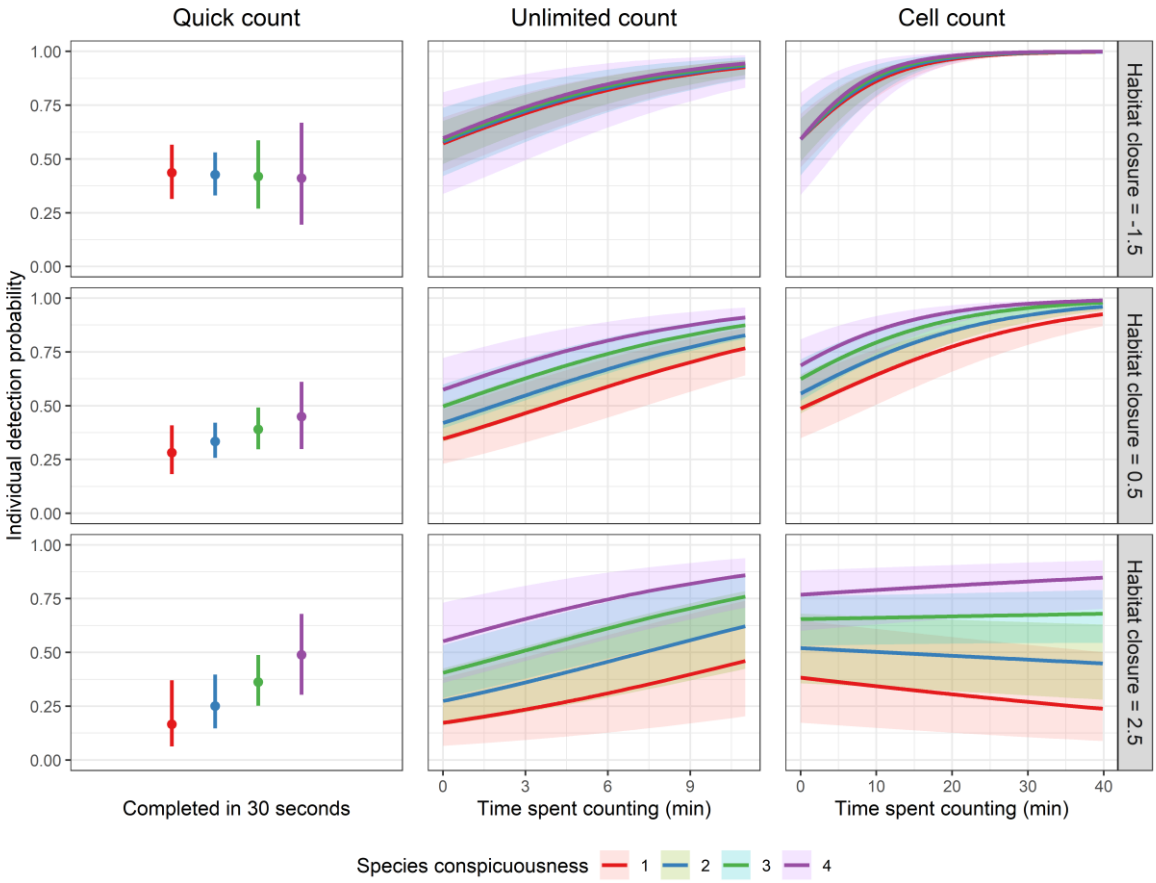

Figure 3: Predicted detection probability depending on species conspicuousness (1 = least conspicuous; 4 = most conspicuous), habitat closure (-1.5 = most open habitats; 2.5 = most closed habitats), the counting method and the time spent counting. Predictions were made for a quadrat containing 100 individuals of the species of interest, located in the fifth position in the round for an observer who rated his/her level of experience in botany at 2.5 out of 5. Lines represent the predicted mean detection probability, and the shading represents 95% confidence intervals.

252

253 Observers achieved higher detection probability with the cell count than the unlimited count,
254 which yielded higher detection probability than the quick count. However, this came at the
255 cost of a major increase in the time spent counting. The counting time per quadrat was fixed
256 at 30 seconds for the quick count, and the mean counting time averaged over all sessions and
257 observers was 1.8 minutes (ranging from 0.3 to 5.7) for the unlimited count and 8.2 minutes
258 (from 0.9 to 21.6) for the cell count. While the time spent increased, the slope between
259 detection probability and the other variables (e.g. observer experience, vegetation closure)
260 was not reduced. Some variables had roughly the same correlation with detection probability
261 regardless of the counting method (e.g. true density), while others had an even steeper slope
262 (e.g. experience in botany).

263 The model predicts that reaching a detection probability over 0.95 (i.e. 'almost perfect
264 detection') is only possible in a very open habitat (typically with <7% vegetation cover and
265 median vegetation height <1 cm) no matter the species' conspicuousness. In a less open
266 habitat (e.g. vegetation cover <90%, median vegetation height <6 cm), the target species must
267 be very conspicuous, i.e. a plant above 20 cm in height or 10 cm but with colourful flowers.

268 Regardless of the species and habitat conditions, reaching this detection probability requires
269 using the cell count, a true density below 100 individuals per quadrat and a counting time per
270 quadrat of at least 20 minutes.

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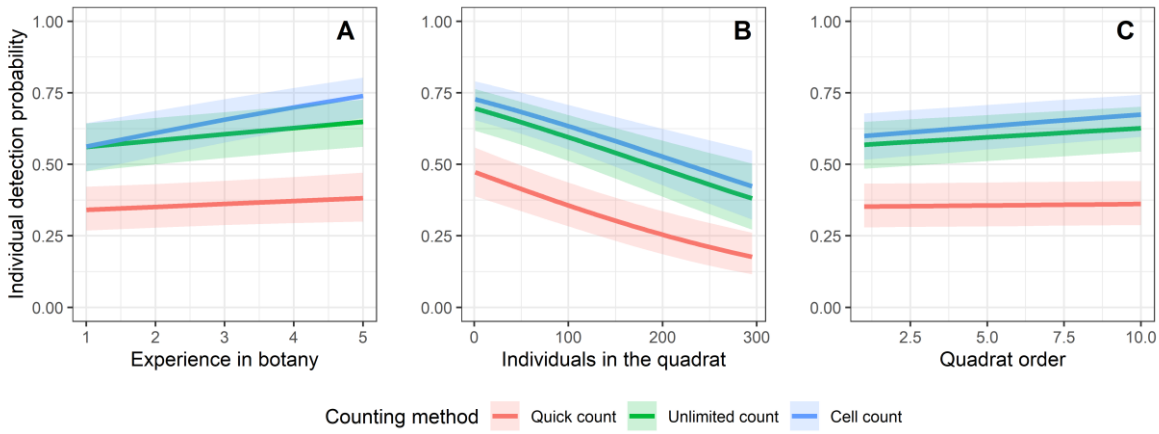

Figure 4: Predicted detection probability of individuals depending on the experience in botany of the observer (A), the true density (B) and the position of the quadrat in the round (C). The other variables included in the model are held constant at the following levels: species conspicuousness = 2, habitat closure = 0, quick count time = 0.5 min, unlimited count time = 3 min, cell count time = 3 min. Lines represent the mean detection probability of individuals, and the shading represents 95% confidence intervals.

272

273 Substantial variation in detection probability between field sessions remained unexplained by
274 the model, even after controlling for species and habitat characteristics, as the variance of this
275 random effect was ${\sigma_{session}}^2 = 0.58$. There was also unexplained variation between quadrats
276 (${\sigma_{quadrat}}^2 = 0.42$). The unexplained between-observer variation was the smallest, with a

variance of ${\sigma_{observer}}^2 = 0.16$. The proportion of variance explained by the model was ${R^2}_{GLMM(c)} = 0.35$, and the proportion of variance explained by the model's fixed effects was ${R^2}_{GLMM(m)} = 0.12$. In other words, detection probability remained heterogeneous even after considering the effect of the seven variables included in the model and accounting for unexplained inter-session, inter-plot and inter-observer variance.

Discussion

This study provides the first estimate of the detection probability of individuals in plant counts, using a detection experiment with a cross-taxa and cross-habitat design. Our results demonstrated that detection is always imperfect when counting plants, even in small (1 m²) quadrats with unlimited counting time. Detection probability was also highly heterogeneous and varied with the species, habitat, observer and counting method. Methods involving more survey time increased the mean detection probability, but did not achieve perfect detection, nor did they reduce the effect of the ecological and observational variables on detection probability. Even after controlling for the effects of all the variables, detection remained highly heterogeneous. We, therefore, argue that plant ecologists should not rely on the use of raw counts and should always take into account detection issues when studying plant populations.

1. Evidence for imperfect detection of plants

In our experiment, while using common plant counting methods (Reisch et al., 2019), not a single observer detected all the individuals present in the quadrats of a field session. Even

298 with counts unlimited in time, the predicted detection probability in average conditions (i.e.
299 for the average values of all variables included in the model with the unlimited count) did not
300 exceed 0.59 (95% confidence interval: 0.50–0.67). Thus, our results not only confirm that
301 imperfect detection is the rule when counting plant individuals, but show that detection
302 probability can even be relatively low. While these values may be surprising, detection issues
303 have been previously documented for plants when searching for a species (e.g. Chen et al.,
304 2013), and a recent study conducted on large quadrats (20x20 m) suggested that imperfect
305 detection may also occur for plant individuals (Hauser et al., 2022). Furthermore, the
306 detection probabilities we found for plants are higher than those commonly found for animals.
307 For example, detection probabilities of 0.04–0.32 have been found in bird point counts (Royle,
308 2004), 0.07–0.41 for lizards counted along transects (Kéry et al., 2009), 0.06–0.41 for
309 salamanders counted along stream banks (Dodd & Dorazio, 2004), and 0.06–0.34 for
310 freshwater mussels searched on riverbeds (Carey et al., 2019). Nonetheless, this does not
311 discount that detection remains far from exhaustive for plants and thus should not be
312 overlooked.

313

314 **2. Effects of the ecological variables**

315 Our results showed that species conspicuousness positively affects detection probability,
316 while habitat closure negatively affects it. This general result was expected, as it has also been
317 observed for plants in occupancy and time-to-detection studies (Alexander et al., 2012;
318 Garrard et al., 2008; Hauser et al., 2022). In open habitats, detection probability was the same
319 regardless of the species' conspicuousness. In contrast, differences between species appeared
320 with increasing habitat closure, reaching up to a 0.42 difference in detection probability

321 between the extreme levels of species conspicuousness in the most closed habitats (estimated
322 difference for the unlimited count with 3 min counting time). This shows that habitat closure
323 drives the detection probability of plant individuals more than species conspicuousness.

324 Furthermore, the negative effect of the density of individuals on detection probability was
325 unexpected in its magnitude (e.g. a predicted detection probability of 0.67 in a quadrat with
326 20 individuals to 0.37 in a quadrat with 300 individuals, using the unlimited count in average
327 conditions) and occurred whatever the counting method. This phenomenon has never been
328 reported to our knowledge, even for animal taxa, but may be due to the Weber–Fechner law
329 regarding how humans perceive numbers (see Nieder, 2020, for a summary). Indeed, studies
330 on human cognition have shown that it becomes increasingly difficult to estimate the number
331 of items in an area as the number grows, and that there is a general tendency to
332 underestimate it. It is plausible that this phenomenon seldom applies to animal counts, as
333 these do not usually involve counting immobile items in high density as is the case for plants.

334

335 **3. Observer effect**

336 The experience in botany of observers had little effect on detection probability compared to
337 the ecological variables and the counting time. In average conditions, the difference in
338 detection probability between novices and the most experienced observers was 0.04 for the
339 quick count, 0.09 for the unlimited count and 0.17 for the cell count, and confidence intervals
340 largely overlapped except for the cell count (Appendix 6). The advantages of having
341 experience in botany when searching for individuals of a target species may be the ability to
342 more quickly identify an individual once it has been detected, to make fewer identification

errors, and possibly to survey the study area more efficiently by knowing where to search for the species (Archaux et al., 2006). These advantages were probably minor in our experiment, given that the observers were trained to identify the target species before starting the counts, all species were easy to identify, and an efficient surveying strategy was of little use in 1x1 m quadrats. In this context, the observers' task can be seen as searching for small objects in a noisy environment, and it seems plausible that their performance was mainly driven by their ability to stay focused during a repetitive task, their motivation, or their fatigue during the experiment. However, larger differences between observers might have been observed if we had involved participants with recent experience in searching for the target species (Hauser et al., 2022) due to the well-known 'search image' effect, whereby foraging predators improve their probability of detecting the type of prey they are trained to detect (Ishii & Shimada, 2009). If this effect indeed applies to plant counts, population trends estimated from raw counts will be biased if the same observers always make the counts, as their detection probability will increase with time.

4. Comparison of the counting methods

As expected, increasing the survey effort, measured by the time spent counting, massively increased detection probability (Archaux et al., 2006; Hauser et al., 2022). In average conditions, the estimated detection probability was 0.36 for the quick count (30 sec counting time), 0.54 for the unlimited count (1.8 min average counting time) and 0.73 for the cell count (8.2 min). In addition, for the unlimited and cell counts, observers spending more time counting achieved higher detection probability (e.g. for the unlimited count in average conditions, detection probability increased from 0.50 at 1 min to 0.83 at 10 min).

366 The gain in detection associated with counting individuals per cell instead of the whole
367 quadrat could be due to two reasons: the cells force the observer to survey the whole surface
368 area of the quadrat uniformly, and also might reduce uncertainty whether or not an individual
369 has already been counted, which frequently occurs when individuals are close to each other.
370 However, contrary to our expectations, increasing survey effort did not reduce the effect of
371 the ecological variables on detection probability, the most striking being the density of
372 individuals, which had an almost identical effect on detection probability for all three counting
373 methods.

374

375 **5. Unexplained variance and implications of detection probability heterogeneity**

376 Of the random effects included in the model, the most unexplained variation in detection
377 probability was at the inter-session and inter-quadrat levels. Since we only measured three
378 synthetic variables to characterise the differences between sessions and quadrats (species
379 conspicuousness, habitat closure and true density), we expected that unexplained variance
380 would remain at these levels. For example, we did not measure colour and height
381 heterogeneity, for the habitat or for the target species, although these variables certainly
382 impact detection probability (Garrard et al., 2013; Hauser et al., 2022).

383 The fact that the unexplained inter-observer variation was the lowest of the random effects is
384 an encouraging result for future studies, as it means that there are few differences in
385 detection probability between observers compared to the differences between species or
386 over a habitat closure gradient. Yet overall, more than half (65%) of the variance in detection
387 probability remained unexplained by our model. Thus, observers had heterogeneous

detection probability between quadrats even after controlling for several variables, which suggests that factors varying over time for each observer, such as concentration or motivation, probably explain a large part of the variation in detection probability.

6. Recommendations for counting plants

Our study shows for plants what has been documented many times for animals: that detection probability of individuals is less than one and is usually heterogeneous in space and time, whatever the counting method and survey effort. Using counts affected by imperfect detection as a proxy for the density of individuals to make comparisons over time and space is only valid under the hypothesis of constant detection. Our findings show that multiple ecological and observational variables cause heterogeneity in detection probability. While observational variables can be standardised, it seems unlikely that ecological variables (i.e. species conspicuousness, habitat closure and true density of individuals) will be constant in any study, even those conducted at limited temporal and spatial scales. Furthermore, trying to achieve almost perfect detection (i.e. >0.95) by increasing survey efforts to fix the issue of detection heterogeneity seems unrealistic given the resource constraints of most ecology and conservation studies. We therefore argue that temporal or spatial trends in plant populations should not be estimated using raw counts, as they could then reflect a trend in ecological variables rather than the population's fate.

Several methods can be used to estimate individual detection probability and thus estimate unbiased population sizes or trends (e.g. Lebreton et al., 1992; Buckland et al., 2001). In particular, the N-mixture method uses repeated counts of unmarked individuals on sampling

410 units (e.g. quadrats or sites) to estimate detection probability from the differences in the
411 number of individuals detected between counts (Royle, 2004). With this method, estimates of
412 population sizes or trends are more precise if detection probability is high and homogeneous
413 in time and space, if the mean density of individuals is high, and if the sample size, i.e. the
414 number of sampling units and count repetitions, is high (Royle, 2004; Ficetola et al., 2018).
415 Thus, for studies using the N-mixture method, no matter the studied population and the
416 counting method used, we recommend setting a fixed counting time, as leaving it to the
417 observer's choice will add heterogeneity to detection probability, resulting in less precise
418 estimates.

419 In terms of allocating survey effort, there are three options: use a more time-consuming
420 counting method to increase detection probability, make more count repetitions, or survey
421 more sampling units. The only way to be sure of the optimal design is to conduct a pilot study
422 to estimate the mean density of individuals and their detection probability in the population
423 of interest. However, Ficetola et al. (2018) have shown that when detection probability is
424 above 0.10, the most advantageous strategy is usually to make three count repetitions and to
425 increase the number of sampling units as much as possible. Conversely, when detection
426 probability is below 0.10, it is better to increase it by using a more time-consuming counting
427 method or to increase the number of count repetitions. Thus, we recommend counting
428 individuals in quadrats without cells with a fixed short counting time (e.g. 0.5–1 min), as this
429 method will yield a detection probability above 0.10 for most species and allow the most
430 sampling units to be surveyed. More time-consuming counting methods should be used only
431 if the study population grows in a very closed habitat and individuals are inconspicuous, or if
432 travel costs resulting from increasing the number of sampling units is a strong constraint.

Acknowledgements

We would like to warmly thank the 167 volunteers (Appendix 9) who participated in the experiment, including during the preliminary tests. We also thank the volunteers who helped organise the field sessions by finding study sites or participants, and Virginie Pons for her help in transcribing the data.

Authors' contributions

J.P., A.B., A.C. and G.P. conceived the study and designed the methodology; J.P. and G.P. organised and conducted the field sessions; J.P. analysed the data and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Data availability statement

All data and code used for this work are available from the GitHub repository: https://github.com/JanPerret/Individual_detection_in_plant_counts

Conflict of Interest statement

All authors declare no conflict of interest.

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