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Tracking rodent-dispersed large seeds with Passive Integrated Transponder (PIT) tags

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Summary

1. Seed dispersal, a critical phase in the life history of many plants, is poorly understood due to the difficulty of tracking and monitoring dispersing seeds until they reach their ultimate fate. Scatter-hoarding rodents play a substantial part in the seed dispersal process of many plant species, however, existing tracking methods do not allow seed monitoring without risk of influencing the hoarding process and seed fate.

2. Here, we describe and test the use of Passive Integrated Transponders (PIT) tags inserted into seeds for the tracking and monitoring of large seeds dispersed by rodents. Unlike other tagging methods, PIT tagging combines the advantages of leaving no external cues and being readable without disturbance of caches. Rodents cannot remove these tags.

3. We evaluated the performance of PIT tagging through a series of trials with *Quercus* acorns dispersed by rodents, both in North America and in Europe, with equipment from different manufacturers. We quantified effects of tagging on seed removal and caching, cache pilferage and seed germination, by comparison between PIT-tagged and untagged acorns. We evaluated the detectability of buried tags to researchers.

4. Minimal effects of PIT tagging on seed removal, caching, pilferage and germination were found. Buried PIT tags were retrieved with high reliability by naïve researchers, even at burial depths up to 30 cm. Identification codes could be read even when multiple tags were buried at a single location, as in larder hoarding.

5. The method was successfully applied in two field studies of dispersal of *Quercus palustris* and *Q. rubra* acorns by Eastern grey squirrels *Sciurus carolinensis* in North America, and *Q. robur* acorns by Wood mice *Apodemus sylvaticus* in the Netherlands. The proportion of seeds recovered was comparable to that in studies using traditional thread tags.

6. We conclude that PIT tagging is a particularly suitable method for tracking and monitoring of seeds dispersed by scatter-hoarding rodents. PIT tagging solves most of the main problems generally encountered when following the fate of rodent-dispersed seeds over time.

Key-words: acorn, hoarding, passive integrated transponder, PIT tag, *Quercus*, rodents, seed dispersal, seed tracking

Introduction

Seed dispersal is a major determinant for plant regeneration (Van der Pijl 1972; Lemke, Von der Lippe & Kowarik 2009). The majority of large-seeded trees, such as oak, chestnut, hickory, pine and many palm species, in temperate, sub-tropical and tropical forests rely on seed hoarding by granivorous mammals and birds for primary and/or secondary seed dispersal (Howe & Smallwood 1982; Jansen, Bongers & Hemerik 2004; Forget *et al.* 2005). These so-called 'scatter hoarders' store large numbers of seeds, individually or in small quantities throughout their home range, serving as food supplies during periods of food scarcity (Morris 1962). The shallow cache sites used by scatter hoarders are often ideal for both seed storage and germination. Thus, when animals fail to recover some of the cached seeds, the latter are likely to establish as seedlings (Vander Wall 1990; Jansen & Forget 2001; Steele & Smallwood 2002; Forget *et al.* 2005). Scatter hoarders can move large numbers of seeds in a relatively short time span, sometimes over considerable distances (Smith & Reichman 1984; Price & Jenkins 1986; Vander Wall 1990; Steele & Smallwood 2002).

Whereas many studies have attempted to quantify the role of scatter-hoarding animals in seed dispersal and tree regeneration, few have been able to actually estimate the proportion of scatter-hoarded seeds germinating and establishing. A major reason is that scatter-hoarded seeds are often repeatedly recovered and re-cached before they reach their ultimate fate (Vander Wall, Kuhn & Beck 2005; Jansen *et al.* 2012). Existing tagging methods, such as coloured threads or flagging tape, inserted magnets or metal objects and radio isotopes (Forget & Wenny 2005) are not well suited for tracking and monitoring

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seeds beyond their initial cache, either until the seeds die or until they germinate and establish as seedlings. One major concern is that these methods present cues to rodents that can increase cache dynamics (e.g. increased re-caching, increased pilferage) and thus bias ultimate seed fate. Internal tags and radio isotopes typically require disturbance of the cache to identify the individual seed, thus producing digging traces that rodents use to locate and pilfer cached seeds (Murie 1977; Guimaraes *et al.* 2005). Likewise, external visual tags may also increase the likelihood of rodents locating cached seeds (Hirsch, Kays & Jansen 2012). Thirdly, traditional tagging methods often result in a considerable increase in seed weight, while seed mass is known to affect seed removal and dispersal (e.g. Jansen *et al.* 2002; Jansen, Bongers & Hemerik 2004).

Here, we describe a new technique for tracking the movement of animal-dispersed seeds that is free of these constraints: internal tagging of seeds with Passive Integrated Transponder tags, henceforth PIT tags. PIT tags are widely used to individually tag animals in husbandry and wildlife conservation (e.g. Elbin & Burger 1994; Hewitt *et al.* 2010; Hoy, Murray & Tribe 2010). In ecology, PIT tags have been used to study the movement and behaviour of freshwater fish (e.g. Greenberg & Giller 2001; Cucherousset *et al.* 2005), and for animal monitoring and studies of population dynamics (reviewed in Gibbons & Andrews 2004). In this article, we provide a technical description of the method, and summarize potential advantages and disadvantages for studying removal, dispersal and caching of seeds. Then, we present results from greenhouse and field experiments that evaluate the suitability of the technique for tracking seed dispersal, using acorns (*Quercus* spp) in two different study systems. Results of a full field study using this method are presented in a companion paper (Steele *et al.* 2011).

Technical description

A passive integrated transponder (PIT) tag is an electronic microchip connected to an electric resonance circuit that acts as a receiving and/or transmitting antenna, encased in a biocompatible glass cylinder (Fig. 1) (Jansen & Eradus 1999). Each PIT tag is programmed with a unique alphanumeric code, permitting individual identification with a Radio Frequency Identification (RFID) transmitter-receiver (Gibbons & Andrews 2004). The RFID reading device generates a carrier radio wave, while an antenna system attached to the reader generates an electromagnetic field that prompts the transponder to send back its code, which is then received by the antenna and interpreted by the RFID reader (Bonter & Bridge 2011). The PIT tags do not require an internal power source, and can work indefinitely. PIT tags are available in various sizes, ranging from 4 × 34 mm down to as small as 1·5 × 7 mm, and weighing just 0·05 g, small enough to be inserted into seeds. PIT tags typically have a read range of about 25–60 cm (Fuller *et al.* 2008), large enough to detect seeds hoarded underground. This range can be extended to 1–2 m with customized antenna and tag designs (Cucherousset *et al.* 2005). Generally, larger tags will have larger detection ranges.

Fig. 1. Two sizes of PIT tags (in cm) (Trovan Ltd.)

Performance tests

We evaluated the performance of PIT tags for tracking seeds in two study systems: acorns of Pedunculate oak *Quercus robur* dispersed by Wood mice *Apodemus sylvaticus* in the Netherlands, and acorns of Red oak *Q. rubra* and Pin oak *Q. palustris* dispersed by Eastern grey squirrels *Sciurus carolinensis* in Pennsylvania, USA. We used the following criteria for this evaluation: (i) no effect on seed mass, which is important because individual dispersal distance and fate are affected by seed mass (e.g. Jansen *et al.* 2002; Jansen, Bongers & Hemerik 2004; Pons & Pausas 2007), (ii) no effect on germination and seedling growth, (iii) negligible effect on animal behaviour related to scent and modification of the seed and (iv) high rates of recovery, that is a low chance of missing seeds within the search area.

In the Netherlands, we prepared experimental acorns by drilling a small hole of 2 × 12 mm in the basal half of each acorn (using a wood drill), inserting a single glass-encapsulated PIT tag (ID100A, 2 × 11·5 mm, 125 kHz, Dorset Identification, Netherlands), sealing the hole with scent-free wax (Entwas, Asepta BV, Netherlands) and polishing the seed with an odourless cloth to remove all traces of wax on the seed shell. We wore scent-free gloves during all seed handling. The entire tagging process takes less than a minute per seed. The reading equipment consisted of a high-performance handheld reader (GR-250, Dorset Identification, Netherlands) and a flat-panel antenna system (LID-650, Dorset Identification, Netherlands) optimized to work with these PIT tags. The reader continuously displays tag code data received by the antenna, and alerts the operator whenever a tag is detected (as in Cucherousset *et al.* 2005). This system allows the simultaneous reading of multiple tags, which is important when caches can contain more than one seed.

In Pennsylvania, we used similar-sized PIT tags (1·5 × 12·5 mm, 134·2 kHz; Model HPT12, Biomark Corporation, Idaho, USA) with a Destron-Fearing reader and hand-held loop antenna (Model FS2001F-ISO, Biomark Corporation, Idaho, USA). Acorns of pin oak and red oak were prepared and tagged in a similar manner to that described above, sealing the hole first with odourless wood filler (Elmer's Products Inc., Ohio, USA), allowing it to dry and then disguising the sealed hole with a small patch of the filler similar in colour to the shell of the acorns (colour varies considerably with oak species). Careful closure is important in this system, because any indication that the shell of an acorn is compromised will likely result in selective consumption rather than caching of an acorn by rodents because the animals are highly sensitive to seed perishability (Hadj-Chikh, Steele & Smallwood 1996; Steele, Hadj-Chikh & Hazeltine 1996).

To evaluate effects of PIT tagging on seed mass, we randomly selected and numbered 60 Pedunculate oak acorns in the Netherlands, and 60 Pin oak and 59 Red oak acorns in Pennsylvania from composite samples from 3 to 5 trees of each species. We weighed each acorn with a precision balance, inserted a PIT tag, reweighed the acorn after drying of the glue and/or filler, and compared the weights with pairwise t-tests.

For Pedunculate oak in the Netherlands, we report results from a 2-year field study where nearly 1200 PIT-tagged acorns were offered to wood mice at twelve different locations in October 2010 and October 2011. Hoarded acorns were subsequently relocated and followed through time until July the following year, at which time caches were recovered and long-term seed fate was established. In addition, we experimentally tested the effects of PIT tagging on seed germination and seedling establishment, by comparing the proportion of acorns germinating and seedling growth between 60 tagged and 60 untagged acorns of Pin oak in Pennsylvania. Acorns were germinated in 1-litre plastic containers by filling the containers with dampened paper towels and nesting the acorns within the towels along the sides of the container so their germination progress could be regularly observed. This approach allows acorns to grow well up to 12 weeks or more and allows assessment of seed performance without the further confounding effects of soil nutrients. Equal numbers of PIT-tagged and untagged acorns were alternately placed around the edge of each container. Paper towels were moistened daily. Seed germination was initiated on July 23, 2010 and all seedlings were harvested on August 13, 2010. For each seedling, we measured the radicle length, epicotyl length and number of leaves. We tested for differences with Chi-squared tests (germination) and t-tests (seedling size).

We assessed effects on animal behaviour in the Netherlands, by presenting differently marked acorns to wood mice and recorded their handling and removal. We tested two wire-marking techniques; wire glued to the acorn and wire stitched through the acorn, and one type of internal tag marking; a 5 × 3 mm tag inserted into the acorn (and the hole sealed with scent-free wax). Here, the tag was a magnet rather than a PIT tag, but the treatment was the same otherwise. We also report preliminary findings from a field study using PIT tags in the same area. In a separate study in Pennsylvania, we presented habituated, free-ranging Eastern grey squirrels in a semi-natural park setting with PIT-tagged and untagged acorns (Steele *et al.* 2011) to follow patterns of seed fate before and after cache owners were removed from the site.

To assess the accuracy of cache retrieval by researchers, we buried PIT-tagged acorns of Pedunculate oak in an open grassy field in the Netherlands and had a naïve researcher attempt to recover them. We individually buried 12 PIT-tagged acorns at each of 4 depths: 5 cm, 10 cm, 15 cm and 30 cm, and determined what proportion was retrieved by the test person. We also buried 12 tagged acorns at 10 cm depth randomly scattered across a grassy field of 1000 m^2 and then had a test person, who was unaware of the cache locations, recover them. We also report here some data on cache retrieval for the long-term field study we performed in the Netherlands. Likewise, in Pennsylvania, we buried 12 PIT-tagged acorns of Red oak at each of 3 depths: 5 cm, 10 cm and 15 cm and had an observer recover them. Finally, we tested for the maximum detection range of both systems, measuring maximum distances at which 12 individual PIT-tagged acorns were detected when placed on the soil surface.

Results

EFFECTS ON SEED MASS

Passive integrated transponder(PIT) tagging resulted in a significant, yet minor increase in seed mass in two of the three species (Fig. 2). Mean mass of individual acorns changed from

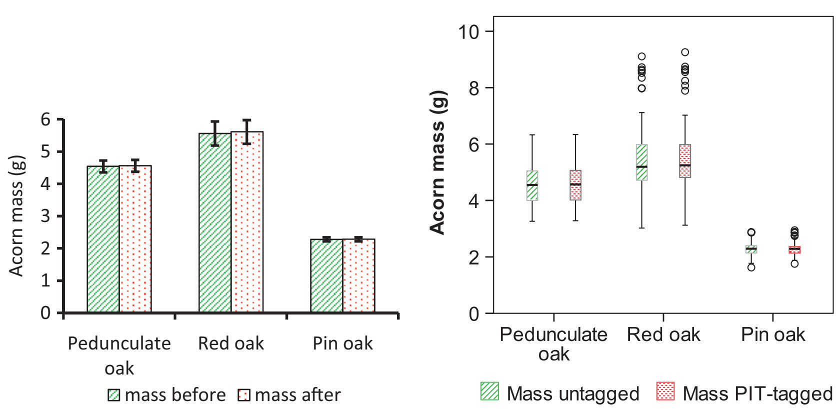

Fig. 2. Effects of PIT tag insertion on seed mass for Pedunculate oak (N = 60), Red oak (N = 59) and Pin oak (N = 60). Error bars represent 95% confidence intervals.

4·54 g (N = 60, SD = 0·70) to 4·56 g (SD = 0·70) after PIT-tag insertion in Pedunculate oak (Paired t-test; $t = 16{\cdot}25$, d.f. = 59, $P < 0{\cdot}001$), from 5·56 g (N = 59, SD = 1·44) to 5·61 g (SD = 1·41) in Red oak ($t = 5{\cdot}32$, d.f. = 58, $P < 0{\cdot}001$) and from 2·280 g (N = 60, SD = 0·251) to 2·281 g (SD = 0·243) in Pin oak ($t = 0{\cdot}10$, d.f. = 59, $p = 0{\cdot}9$). However, seed mass did not significantly differ between randomly selected PIT-tagged and untagged acorns from the same source, neither in Pedunculate oak (Student's t-test: $t = 0{\cdot}013$, d.f. = 118, $P = 0{\cdot}99$) nor in Red oak ($t = 0{\cdot}198$, d.f. = 116, $P = 0{\cdot}84$).

EFFECTS ON GERMINATION AND SEEDLING ESTABLISHMENT

In Pedunculate oak, a field study showed that PIT-tagged acorns readily germinated and established as seedlings. Of nearly 1200 PIT-tagged acorns offered to and hoarded by wood mice in October 2010 (N = 589) and October 2011 (N = 588), 833 (70·8%) were later retrieved within the search area. Of these, 114 (13·7%) still remained in July the following year. Of these last 114 acorns that were not recovered by the wood mice, only 5 (4·4%) had died from a fungal infection while all others had germinated (95·6%). Finally, 72 (63·2%) of the PIT-tagged acorns that had germinated, emerged and established as seedlings.

In Pin oak, a greenhouse experiment showed a slight effect of PIT tagging on germination but little effect on seedling growth. We observed initial germination (radicle growth > 1 cm) in 45 (75·0%) of the 60 tagged acorns and 55 (91·7%) of the 60 untagged acorns ($\chi^2 = 5{\cdot}255$, $P = 0{\cdot}02$; Fig. 3a). Continued germination (plumule emergence) was observed in 39 (65·0%) and 53 (88·3%) of the tagged and untagged acorns respectively ($\chi^2 = 8{\cdot}382$, $P = 0{\cdot}004$; Fig. 3b). We noted that in these nearly 1-year-old acorns, which appeared otherwise sound, traces of fungus or mould were found in 36 of the tagged and 0 of the untagged acorns. This suggests that in older acorns, tagging may predispose them to lower germination success by allowing colonization by pathogens. Thus, care should be taken to use newly collected seeds and to minimize contamination when preparing tagged nuts. Under normal circumstances PIT tagging would occur at the time of acorn maturation and if the acorn is well sealed, mould and fungus are unlikely to penetrate the cotyledon.

Among the tagged and untagged Pin oak acorns successfully exhibiting aboveground seedling growth, we observed nearly identical measures of seedling performance 6 weeks after planting; including mean number of leaves [tagged (N = 39): mean ± SD; 6·2 ± 2·3; untagged (N = 53): 6·0 ± 2·7; Welch's t-test = 0·342, $P = 0{\cdot}73$; Fig. 3c], mean epicotyl height (tagged: 17·6 ± 5·7 cm; untagged: 18·7 ± 5·8 cm; $t = -0{\cdot}973$, $P = 0{\cdot}33$; Fig. 3d) and mean radicle length (tagged: 20·6 ± 6·3 cm; untagged: 20·7 ± 7·2; $t = -0{\cdot}083$, $P = 0{\cdot}93$; Fig. 3e).

EFFECTS ON ANIMAL BEHAVIOUR

In the Netherlands, wire-marking techniques did not work well with wood mice. The majority of wire-marked acorns (55%, N = 76) was not removed from the plot at all by wood mice, and of the acorns that were handled by wood mice, 84% was gnawed off the wire and only the wire was retrieved. In contrast, all magnet-marked acorns (N = 45) were removed from the seed plot within one night. Of these, 19 (42%) were retrieved within the search radius of 30 m, while the other acorns had probably been dispersed outside the search area. In 10 cases (53%), acorns were found intact while in the other 9 cases magnets were retrieved together with acorn shell remains, indicating consumption at the retrieval site. Our field study with PIT-tagged acorns (12 sites, 49 acorns per site, N = 588) provided similar results to those from magnet-tagged acorns. All PIT-tagged acorns were removed within one to three nights and were often retrieved intact in shallow individual caches throughout the search area or as exposed PIT tags accompanied by acorn shells, indicating local consumption.

In Pennsylvania, animals never rejected tagged acorns, but instead either ate or cached them within sight. After consumption, PIT tags were often dropped at the feeding site and easily recovered among the feeding debris. Immediately after the animal cached an acorn, we mapped the cache location and verified the presence of the acorn in the cache site. We were then able to revisit cache sites almost indefinitely and monitor the presence of the cache without disturbing the cache in any

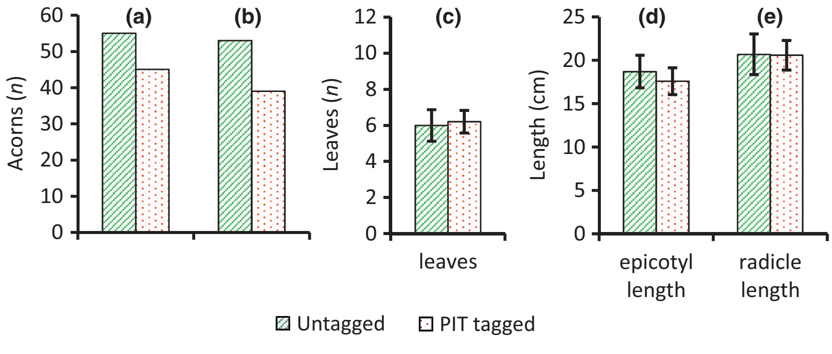

Fig. 3. Effects of PIT tagging on germination (a; N = 60), plumule emergence (b; N = 60), number of leaves produced (c; N = 55 for untagged and N = 39 for PIT tagged), mean epicotyl length (d; N = 55 for untagged and N = 39 for PIT tagged) and mean radicle length (e; N = 55 for untagged and N = 39 for PIT tagged) for Pin oak acorns. Error bars represent 95% confidence intervals.

manner. When an acorn was removed, either by a pilfering conspecific or by the cache owner, an obvious pit was observed and the PIT tag was not detectable. When the acorn was eaten at the site, observers often recorded acorn shell fragments and the intact PIT tag. Steele *et al.* (2011) used this technique to follow the fate of acorn caches and assess pilfering rates of natural caches when cache owners were removed from their home ranges.

RETRIEVAL SUCCESS

Using the Trovan system in the Netherlands, all seeds artificially cached at 5, 10 and 15 cm depth were detected and read by the naïve researcher. At 30 cm depth, 90% of the acorns were detected and read. The test person also recovered all acorns that we artificially scatter hoarded (at 10 cm depth) within 6 min, without need to repeatedly search the same area. The maximum depth of detection for 12 tagged acorns ranged between 27·8 and 34·2 cm (31·43 ± 1·70), and the system was capable of detecting and reading up to 6 PIT-tagged acorns cached together, and of detecting caches with up to 16 PIT-tagged seeds. Results from a field study in a natural forest area in the Netherlands, where nearly 1200 PIT-tagged acorns were offered to and hoarded by wood mice, showed a retrieval success of 70·8% (N = 833) within a search radius of 45 m from the source location. Likewise, using the Biomark system in Pennsylvania, all PIT-tagged acorns were detected and read at all three depths. Maximum depth of detection for 12 tagged acorns of Red oak ranged between 18·0 and 31·5 cm (23·75 ± 4·77), which is enough to also detect seeds that have been taken into an average-size burrow system (Jennings 1975).

Discussion

Assessing the ultimate fate of seeds dispersed by rodents requires tracking and monitoring of seeds without animals severing the tags, and without tags presenting cues to pilferers, as the latter may accelerate or seriously alter cache dynamics (e.g. increased re-caching, increased movement of seeds and increased risk of predation on seeds) (Guimaraes *et al.* 2005; Hirsch, Kays & Jansen 2012). Here, we describe how insertion of PIT tags allows the non-invasive measurement of removal, dispersal and ultimate fate of seeds dispersed by scatter-hoarding rodents. We show that i) insertion of PIT tags in acorns has a negligible effect on seed mass, ii) PIT tagging hardly influences acorn germination and seedling establishment probabilities, iii) PIT-tagged seeds are treated similarly by seed dispersers as untagged seeds and that the tags are not removed and iv) experimenters' retrieval success of PIT-tagged acorns after dispersal is high.

Although a diverse range of seed-marking techniques has been described in literature (for a review, see Forget & Wenny 2005), none of them possesses the unique combination of advantages listed above. For example, metal- (e.g. Sork 1984), magnet- (e.g. Den Ouden, Jansen & Smit 2005) and radio-isotope labelling (e.g. Vander Wall 1994, 2000) also hardly affect seed mass, but do not allow individual identification of the seed, at least not without disturbing the cache and/or leaving a possible cue for cache detection (e.g. Forget 1990). Similarly, thread marks (e.g. Forget, Munoz & Leigh 1994), wire tin-tags or plastic seed tags (e.g. Xiao, Jansen & Zhang 2006), telemetric thread tags (Hirsch, Kays & Jansen 2012) and VHF radio transmitters (e.g. Tamura 1994; Soné & Kohno 1996) also allow individual identification of seeds, but they may significantly increase seed mass, are frequently severed by rodents, can influence seed predation and germination rates if seeds are pierced for tag attachment, or present cues to cache pilferers (Hirsch, Kays & Jansen 2012). In contrast, PIT-tagged seeds were readily removed from seed plots and cached or consumed by rodents without leaving cues for pilferers, both in the Netherlands and in Pennsylvania. And although a greenhouse experiment with 1-year-old Pin oak acorns showed some effects of PIT-tagging on seed germination, it did not seem to affect seedling growth once the seedling had emerged. We in part selected pin oak acorns for these germination studies because their size is among the smallest for oaks, increasing the potential for a negative effect of the tag on the embryo. It should also be noted that the dormancy period (i.e. cold stratification) of red oak species (section: *Lobatae*) requires older acorns from the previous year to evaluate germination rates. Under normal circumstances, red oaks would be tagged and sealed a few weeks after maturation and germination would begin about 5, 6 months later, thus reducing the probability of rot.

Passive integrated transponder(PIT) tags remain detectable virtually indefinitely, allowing to follow seedlings even after the acorns have rotten away. In Pennsylvania, we have recovered PIT tags at the base of tree saplings more than 4 years after deployment in the field. Although PIT tagging involves piercing of the shell and removal of some cotyledon mass, the tags are entirely inside and the acorns are sealed after PIT-tag insertion. As a result, the long-term impact on seed rot, seedling establishment and growth is likely no worse, and possibly far better, than with other tagging methods that involve piercing of the acorn shell. For example, the metal-tagging method first suggested by Sork (1984) and used extensively by Steele *et al.* (2001) and Moore *et al.* (2007) is generally assumed to have no negative effect on seedling establishment and seedling survival, despite the fact that these brad nails typically leave the acorn exposed to possible pathogen infestation. Moreover, oaks are known to use only a small percentage of the cotyledon biomass for seedling development up to autotrophy (e.g. Bossema 1979; Andersson & Frost 1996). This is also why cotyledon removal by jays after seedling emergence has no adverse effects on seedling growth or development (Bossema 1979; Sonesson 1994; García-Cebrián, Esteso-Martínez & Gil-Pelegrín 2003). Therefore, any negative effects of PIT tagging are likely to show during the early developmental stages of the seedling. That PIT tags do not seem to influence long-term seed fate is further suggested by our 2-year field study in the Netherlands, in which PIT tags did not markedly influence seed survival, seed germination and seedling establishment.

Germination and seedling establishment probabilities for PIT-tagged acorns were similar to probabilities reported for untagged acorns (90–100%; e.g. Shaw 1968). This low apparent impact, combined with the fact that PIT tags do not require internal batteries and thus function indefinitely, illustrates their great potential for studying ultimate seed fate and seedling establishment, particularly when seeds with delayed dormancy must be followed until germination and seedling emergence.

Another advantage of PIT tagging, not evaluated in this article, is the possibility of wiring specific areas to record passage of individual seeds (and animals that carry PIT tags) over a data logger. This provides more robust data than monitoring with remote cameras (e.g. Jansen & Den Ouden 2005), and it requires far less effort. This is particularly useful for studying removal rates of seeds from a central location (e.g. a seed station) or for studying pilfering from a previously created cache. Moreover, PIT tags can be simultaneously used for disperser and seed identification, by not only individually marking seeds, but by also marking the animals that disperse the seeds. Such an approach can allow one to control for independence of individual dispersal events and study the interaction of conspecific scatter hoarders, such as pilfering behaviour in an experimental setting (see Vander Wall *et al.* 2008).

Prices for individual PIT tags vary between $2·50 and $5·50, depending on type and size of the tag. Tags used in this study cost €2·20 (~ $2·75) per tag in the Netherlands and $5·00 per tag (for a purchase over 500 tags) in the United States. Generally, readers and antennae cost around $500 – $1500, again depending on brand, size and qualifications. The customized flat-panel antenna system used in the Netherlands cost around €1050 (~ $1350), while the handheld reader was €700 (~ $900). The Biomark system used in Pennsylvania currently costs $3125 for the advanced reader and loop-antenna system used in this study (cheaper systems < $1000 are available). If compared to thread-, metal- or magnet marking the use of PIT tags is more expensive, however, if compared to telemetric thread tags or VHF radio transmitters this technique is considerably cheaper. Also, since PIT tags function indefinitely, they can be re-used in various studies, lowering the costs per study.

One disadvantage of PIT tags remains the need to closely approach buried tags for detection and scanning, as in tagging with magnets, metal and radio isotopes. In irregular terrain and dense vegetation, where it can be hard or even physically impossible to cover every spot of the search area, PIT tagging can be somewhat labour intensive (it generally takes 1 h to search a forest patch of 25 × 25 m) and may result in more false negatives and lower recovery rates than tagging with thread tags or active radio transmitters, which can be detected from a greater distance. In the temperate forest study systems in which we used PIT tags, however, we achieved higher recovery rates than prior studies using other tags at the same sites, even though the retrieval success for our field study in the Netherlands was negatively influenced by the size of our search area. Our search area covered a radius of 45 m around the seed station, but it is likely that some seeds were dispersed beyond this distance.

We conclude that PIT-tagging is an excellent technique for tracking seed removal, dispersal, re-caching and ultimate seed fate in a variety of field and laboratory situations. PIT tagging solves some of the main problems generally encountered when following the fate of animal-dispersed seeds over time. They provide a reliable, non-invasive and durable seed-marking technique particularly i) in systems where typical seed-dispersal distances are less than about 50 m or in experiments where dispersal is limited to a fixed area, ii) in studies where the focus is not on initial dispersal, but more on re-caching and ultimate fate of seeds and iii) in long-term monitoring projects in which seed germination and seedling establishment are followed.

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