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RESEARCH ARTICLE

Contingent Conclusions: Year of Initiation Influences Ecological Field Experiments, but Temporal Replication is Rare

Kurt J. Vaughn[1,2] and Truman P. Young[1]

Abstract

Interannual variation in experimental field conditions produce variability in the results of experiments monitored over multiple years, termed here "year effects." When experimental treatments are replicated in separate years, interannual variation may influence treatment effects and produce significant treatment by initiation-year interactions. Understanding the frequency and strength of these effects requires initiating identical experiments across years. We conducted a review of literature covering more than 500 experimental articles published in 7 journals between 1966 and 2008. Only 5% of the 276 general ecological field studies initiated experiments in multiple years. This rarity was even more evident in the journal *Restoration Ecology*, in which none of the 173 surveyed experimental studies initiated experiments in multiple years. In contrast, 48% of the 58 field experiments published in an agronomy journal were replicated across years. We found only 17 studies that tested treatment by initiation-year interactions. Despite their rarity, 76% of these studies found significant interactions between treatment and initiation year. We conclude that the results of many ecological field experiments are likely to be contingent on the year in which they are implemented. We discuss the importance of treatment by initiation-year interactions in ecology and restoration, factors that have hindered the inclusion of temporal replication in the past, and some suggestions for the appropriate design and analysis of temporally replicated experiments. We argue for more deliberate investigation of temporal contingency in ecological experimentation, especially in the field of restoration ecology, which may be particularly sensitive to treatment by initiation-year interactions.

Key words: agronomy, annual phenomena, historical contingency, interannual variation, multiple initiation-years, restoration ecology, treatment by initiation-year interaction, year effects.

Introduction

Interannual variations in biotic systems have been widely documented by ecologists, including such dramatic phenomena as masting (Kelly 1994), episodic recruitment (Estes & Duggins 1995), irruptions (Predavec 1994) and epidemics (Caceres et al. 2006), as well as more common variations in the demographies (Suarez et al. 2005; Lee et al. 2007) and behaviors (Callaghan et al. 1997; Rotenberry & Wiens 1998) of plants and animals. This interannual biotic variation often has substantial impacts on interspecific interactions (Riginos & Young 2007; Veblen 2008), the structure of communities (Pitt & Heady 1978; Bakker et al. 2003), and the functions of ecosystems (Sala et al. 1988; Knapp & Smith 2001). Despite widespread recognition of the importance of temporal variation to ecological processes (hereafter, "year effects"), few ecological studies are designed to test how year effects interact with experimental results.

[1] Department of Plant Sciences, University of California, Davis, CA 95616, U.S.A.
[2] Address correspondence to K. J. Vaughn, email kjvaughn@ucdavis.edu

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The outcome of an experiment depends on the conditions under which it is implemented. For example, because uncontrolled variation in field conditions exists at different experimental sites, researchers recognize the importance of testing experimental conclusions across spatial variation. Replicating experiments across multiple sites increases the confidence, generality, and applicability of conclusions (Krebs 1989). By this same logic, testing experimental conclusions across temporal variation by initiating experiments in multiple years also increases the validity of conclusions. Interannual variation in uncontrolled experimental field conditions (e.g. precipitation, temperature, or competitor/predator pressures) can drive differences in results for experiments conducted in different years (Johnson 2002; Young et al. 2005).

The fundamental answers to ecological questions may depend on the year in which the question is asked (Walters et al. 1988). For instance, Bakker et al. (2003) initiated identical treatments in three different years in order to test the contingency of grassland restoration techniques across interannual variation. A subset of these plots was then resampled 9–11 years after plot establishment (MacDougall et al. 2008). Both studies found that their experimental treatments had profoundly different effects in each of the three initiation years, that is, conclusions were contingent on the year of experimental initiation.

We assessed temporal replication across five general ecological journals and two journals representing specialized areas of applied ecological study (agronomy and restoration ecology) in selected years from 1966 to 2008. The goals of this study were to (1) assess the extent to which ecologists replicate experiments across years and (2) assess whether the effects of experimental treatments differ among different years of experimental initiation. We discuss the importance of treatment by initiation-year interactions in ecology and restoration and factors that have hindered the inclusion of temporal replication in the past, and offer some suggestions for the appropriate design and analysis of temporally replicated experiments. We argue for more deliberate investigation of temporal contingency in ecological experimentation, especially in the field of restoration ecology, which may be particularly sensitive to treatment by initiation-year interactions.

Methods

We surveyed all articles published in the journals *Ecology*, the *Journal of Ecology*, *Ecological Applications*, the *Journal of Applied Ecology*, and *Oikos* in 2006. Additionally, we included all articles published in the *Journal of Agronomy and Crop Science* in 2006 and 2007 and *Restoration Ecology* from 2003 to 2008, adding the additional years to increase our sample sizes for the latter two journals. These peer-reviewed journals were selected to cover both a broad range of general ecological topics, and the specialized areas of agronomy and restoration ecology. Because plant establishment can be dependent on interannual variation (Bakker et al. 2003), and both agronomy and restoration focus on the establishment of plant populations or communities, we expected these disciplines to be especially concerned with interannual variation. In order to explore trends over time, we also surveyed all experimental studies published in *Ecology* in 1966, 1976, 1986, and 1996.

First, each article was examined to determine if it described a manipulative field experiment. All articles that did not include a manipulative field experiment were excluded. Each field experiment was then categorized according to the following criteria: (1) type of focal organism (plant, animal, or microbe/other), (2) type of system (terrestrial, freshwater, or marine), and (3) type of parameter manipulated (abiotic, biotic, or both). Finally, we recorded the number of years in which the experiment was initiated, and the number of years the experiment was monitored.

Issues with Experiments Initiated in Multiple Years

While agronomic studies often initiated identical experiments in multiple years, none of the general ecological studies that initiated experiments in multiple years repeated completely identical experiments in more than one year. Experiments in which the design was substantially changed between years were excluded, but experiments with minor changes to their designs were included. For example, a change in sample size, minor changes to some individual treatments, and the implementation of a different set of treatments (as long as more than one treatment overlapped) did not disqualify an experiment from our analysis. An experiment was considered significantly changed in a subsequent year if: (1) any residual effects from manipulations in the previous year were evident or suspected (e.g. Potts et al. 2006); (2) the same site was not used (e.g. Benedetti-Cecchi et al. 2006); (3) a different set of treatments was implemented (e.g. Sweeney & Vannote 1986); or (4) the timing of treatment or methodological practices was substantially changed. For experiments initiated in multiple years that passed these filters, we assessed whether a treatment by initiation-year interaction was reported, and whether or not it was significant.

Finally, we assessed whether the focal ecological phenomenon of the study was of an annual or multiple-year nature. For example, a brood manipulation experiment examining the fledging success of nestlings was considered to represent an annual phenomenon (Haydock & Ligon 1986). If the same experiment had instead focused on the multi-year survival of offspring (or parent) birds following a single brood manipulation, it was considered a multiple-year phenomenon (e.g. Young 1996).

Results

Of the 2,354 total articles examined, 507 were experimental field studies: 276 of these field experiments were published in the five general ecological journals, 173 in *Restoration Ecology*, and 58 in the *Journal of Agronomy and Crop Science* (Table 1).

Of the 507 field experiments included in this survey, 43 (8.5%) repeated nearly identical experiments in multiple years. However, the large majority (28) of these studies were published in the *Journal of Agronomy and Crop Science*, where nearly half (48%) of the field experiments were replicated across years (Table 1). Only 15 of the 276 (5.4%) surveyed experimental studies published in the 5 general ecological journals initiated treatments in more than a single year (Table 1). We found zero examples of field experiments published in *Restoration Ecology* in the surveyed volumes that initiated experiments in multiple years.

There was no significant trend in the proportion of studies initiating experiments in multiple years in *Ecology* through time. No multiple-initiation-year experiments were published in *Ecology* in 1966 or 1976 (out of 11 and 9 total field experiments, respectively). We found 4 multiple-initiation-year experiments of 48 studies (8.3%) published in *Ecology* in 1986, 2 out of 38 (5.3%) in 1996, and 5 out of 73 (6.8%) in 2006.

Experiments Initiated in Multiple Years

Out of the total 43 experiments that replicated experiments across years, only 17 reported treatment by initiation-year

Table 1. Comparison of the number of studies initiated in multiple years, total number of field experiments included in the survey, and the percent of experiments initiated in multiple years across the three disciplines.

	Number with Multiple Initiation-Years	*Studies Included in Review*	*Percentage with Multiple Initiation-Years*
Field of study			
Agronomy	28	58	48.3
Restoration ecology	0	173	0
General ecology	15	276	5.4
Focal organism*			
Plant	5	137	3.6
Animal	10	124	8.1
Microbe/fungus/other	0	15	0
Habitat type*			
Terrestrial	14	199	7.0
Freshwater aquatic	1	45	2.2
Marine	0	32	0
Manipulation type*			
Abiotic	4	96	4.2
Biotic	9	146	6.2
Both	2	34	5.9

* For the general ecological studies, focal organism, habitat, and manipulation type comparisons are also included.

interactions. Of these, 13 (76%) reported at least one significant treatment by initiation-year interaction. Breaking this down further, among the general ecological articles only 6 reported the interaction of treatment and year, 4 of which (67%) reported a significant interaction, whereas in the *Journal of Agronomy and Crop Science* we found 11 studies that reported the interaction of treatment and year, and 9 (82%) found a significant interaction.

Not surprisingly, all 28 (100%) of the agronomic studies initiated in multiple years were plant focused. In contrast, among general ecological studies, animal-focused ecological experiments were more than twice as likely, as a percentage of those studies compared to plant-focused studies, to design experiments initiated in multiple years (Table 1). Terrestrial ecological studies represented 72% of all field experiments and nearly all (93%) of the multiple-initiation-year studies (Table 1).

Forty-four percent of all the surveyed experiments were both initiated in and followed for a single year (Table 2). We found only two (5%) multiple-initiation-year studies that also monitored each experimental initiation for more than a single year (Table 2). This trend may mostly be driven by the fact that

Table 2. Results classified by the number of years in which the experiments were initiated and the number of years that the experiments were monitored.

	Years Monitored				
Years Initiated	*≤1*	*2*	*3*	*4*	*≥5*
1	222	105	46	21	70
2	29	0	2	0	0
≥3	12	0	0	0	0

96% of agronomic multiple-initiation-year studies investigated phenomena of an annual nature (e.g. annual crops). However, this trend is also evident in the general ecological studies where a majority (64%) of multiple-initiation-year experiments investigated phenomena of an annual nature, such as brood manipulation (Haydock & Ligon 1986; Young 1996; Shutler et al. 2006) or pollination experiments (Agren 1996).

When we compared the relative abundance of studies (1) initiated in a single year and monitored for a single year, (2) initiated in a single year and monitored for multiple years, (3) initiated in multiple years and monitored for a single year, and (4) initiated in multiple years and monitored for multiple years, we found that studies both initiated in and followed for multiple years were substantially under-represented (only 2 out of 507; see cells in the bottom right of Table 2).

Discussion

Ecological experiments are rarely replicated across years, despite several examples of strong treatment by initiation-year interactions. Only 5% of all general ecological field studies initiated nearly identical experiments in multiple years. However, our findings further revealed that in the rare cases when field experiments are initiated in multiple years, the results are commonly strongly contingent on the year in which the treatments are implemented. Among the few studies which reported testing interactions between treatment and initiation year, a large majority of both ecological and agronomic studies (75 and 82%, respectively) found significant interactions. In other words, the response(s) to an experimental treatment and the conclusions drawn differed significantly among years.

It is possible that our findings underestimate the true proportion of ecological experiments that initiate experiments in multiple years. Because researchers are less likely to report nonsignificant results (Csada et al. 1996), they might also be less likely to report experimental years with contradictory or nonsignificant results perhaps attributing the "bad" year to a temporal anomaly or some technical detail. We found at least one such example (Svecnjak et al. 2006) and have no way to estimate how often results from multiple initiation-years are unreported. Similarly, our results may overestimate the proportion of multiple initiation-year studies that found significant interactions between treatment and initiation year because researchers may be less likely to report nonsignificant interactions.

The concept of replicating experimental treatments across years to understand how treatment effects interact with year effects is not new (Cochran & Cox 1957). Similarly, the necessity of capturing the ecological consequences of interannual variation through long-term studies is well established (Strayer et al. 1986; Franklin 1989; Likens 1989); long-term experimental studies found in our literature review virtually always followed a single experimental initiation. This gap in long-term, multiple-initiation ecological experimentation is likely to have profound implications if systems with long-lasting effects of establishment are sensitive to the year of experimental initiation. For example, initial conditions have been demonstrated to have lasting effects on community assembly (Samuels & Drake 1997; MacDougall et al. 2008).

Treatment-year interactions are likely to affect any system in which environmental or ecological conditions show substantial interannual variability. Even in a highly variable system, initiation-year interactions will only be detected if treatment outcomes are altered in an observable way across years. Therefore, we might expect temporal replication to be most important for treatments that experience high interannual variation and have high sensitivity to that variation.

Annual phenomena may be particularly sensitive to treatment by initiation-year interactions (Lohmus 2003). Nearly all of the multiple-initiation-year experiments published in the *Journal of Agronomy and Crop Science* investigated annual crop yields. When the phenomenon of interest is inherently annual, multiple-year studies necessarily involve multiple experimental initiations. Correspondingly, a majority of ecological experiments initiated in multiple years (in this survey) examined annual phenomena, such as brood success (Haydock & Ligon 1986; Young 1996; Shutler et al. 2006), pollination (Agren 1996), or annual plants (Kniskern & Rausher 2006). This focus on annual phenomena, which, by their nature, can only be monitored for a single year, may also explain another aspect of our findings: a significant under-abundance of experiments both initiated in multiple years *and* followed for multiple years.

Those treatments investigating strong environmental filters or bottlenecks may also be especially sensitive to treatment by initiation-year interactions. In particular, the conditions in the year of establishment for perennial plants are potentially more influential than conditions in subsequent years (Wedin & Tilman 1993; Milchunas & Lauenroth 1995). It is clear that the establishment phase for plants is often the critical stage for a wide variety of long-lasting ecological phenomena, including niche differentiation (Grubb 1977; Young et al. 2005), competition (Dyer et al. 2000; Rice & Dyer 2001; Harmon & Stamp 2002), and community structure (Grubb 1977; Stampfli & Zeiter 2004). For example, there is considerable evidence that early germination and an early size advantage can increase plant intraspecific and interspecific competitive ability (Dyer et al. 2000; Freckleton & Watkinson 2001; Rice & Dyer 2001; Harmon & Stamp 2002).

The results of this literature review are complicated by the very problem it attempts to highlight. Because so few studies have initiated experiments in multiple years, few statistically rigorous conclusions can be drawn as to what is distinctive about those that do. Nonetheless, here we ask: *Why is temporal replication under-represented in ecological field studies?* We suggest four barriers to the more widespread implementation of temporal replication.

First, short-term funding cycles and publishing pressures create strong financial and temporal pressures for ecologists to implement and publish research over short time frames. Resource pressure can limit the number of ecological experiments that can afford to incorporate temporal replication into experimental design. We found, however, that resource pressures can only partially explain this issue. More than half (56%) of the studies included in this review were monitored for multiple years, with 14% monitored for 5 years or more. This suggests that ecologists are regularly able to commit resources to multiple-year experiments but more often chose to put those resources into following a single initiation year for multiple years.

Second, following a single initiation for multiple years may be considered more informative, technically simple, or cost effective than following multiple initiations for fewer years. If ecological experimentation were a simple compromise between the length of monitoring and the number of possible (annual) initiations, we might expect an equal proportion of long-term monitoring and multiple initiation studies. We do note that the resource intensity associated with the initiation of an experiment in additional years is likely to be greater than the cost of monitoring the same experiment for multiple years.

Third, given a choice between spatial and temporal replication, spatial replication may be a preferred method for incorporating environmental variability into experimental designs. The value of spatial replication, relative to temporal replication, depends on the environmental conditions under investigation. For instance, many soil properties exhibit high spatial variability but low temporal variability at the scale of most field experiments, whereas many variables associated with climatic events show the opposite pattern. Thus, spatial replication may be the preferred method for incorporating some types of "slow" environmental variables (e.g. soil depth), whereas temporal replication may be more effective for other "dynamic" variables (e.g. soil water availability).

Fourth, we suspect that treatment by initiation-year interactions are simply underappreciated by non-agronomic ecologists. These barriers to the more widespread incorporation of temporal replication into ecological experimentation are not insurmountable. While the first barrier, short funding cycles, may represent a real impediment to the inclusion of temporal replication, longer-term funding is increasingly possible (e.g. NSF's LTER and LTREB programs). Interestingly, these special funding opportunities were initiated in part because resource limitations were implicated in limiting the number of long-term ecological studies (Tilman 1989). The latter two impediments are simply the prioritization of limited resources for long-term monitoring or spatial replication over temporal replication.

One practical difficulty with temporal replication is that initiation of identical experiments across years is in conflict with the desire of researchers to improve field methods based on observations of previous year's results. For example, Bakker et al. (2003) found their herbicide application technique unacceptably ineffective during the first year of their experiment. As a result, they changed the practice for the next two years of the experiment, and in so doing achieved better weed suppression for the remainder of their experiment. This form of adaptive management is invaluable for restoration practices and many research projects. However, when year of initiation is itself a factor, any alteration in design, treatments, plot management, or data acquisition methods between years may impact estimates of variance and affect experimental analyses.

By modifying herbicide application practices between years, Bakker et al. (2003) recognized that they confounded year of initiation with plot management technique.

In addition to implementing identical experimental practices, rigorous temporal replication must synchronize the timing of these actions across years. To avoid confounding treatment effects with implementation timing, researchers may choose to implement on the same calendar date each year. However, for some actions it may be more appropriate to follow some important natural or biological phenomena (e.g. planting before the first rain of the season, setting up exclosures after the first observation of a migrant competitor, etc.).

Appropriate statistical analyses of temporally replicated experiments require initiation-year to be included as a factor additional to any other experimental treatments (Cochran & Cox 1957). Care should be used when interpreting a significant interaction between treatment and initiation year, especially because treatment effects may differ across years due to many independently varying environmental factors. Including yearly measures of these variables as covariates in the analyses can help isolate which factors and which years drive this interaction.

If multiple separate experimental initiations are followed for multiple years, the analysis and interpretation of results become more complex. Because each subsequent year's initiation is delayed relative to the first year, a statistically balanced analysis will be restricted to the number of years that all treatments share. Additionally, it may be tempting to attribute treatment by initiation-year interactions to the conditions solely in the year that the experiments are initiated. In fact, each year of initiation differs not only in that year but is also associated with its own unique run of years. Although there may be reason to believe that the year of initiation is the most important of these runs, ideally one would seek to tease apart the effects of the first year from its associated run of subsequent years. One way to isolate these mechanistic factors (and the years in which they have greatest effect) is by including treatments that directly manipulate or simulate interannual environmental variability in the experimental design, such as supplemental watering in dry years.

The disciplines of agronomy and restoration ecology both specialize in the establishment of plant populations or communities, yet have considerably different approaches toward temporal replication. The *Journal of Agronomy and Crop Science* reported temporally replicated experiments in nearly half of the included studies, whereas our survey did not find a single restoration-based field experiment (published in *Restoration Ecology* or in any of the general ecological journals) that initiated experiments in multiple years. This result is particularly striking because many restoration practitioners, if not published restoration ecologists (Bakker et al. 2003; MacDougall et al. 2008), are agonizingly aware that the success of their plantings varies considerably from year to year. We are not the first to advocate for the inclusion of temporal replication in a specialized field of ecology (Walters et al. 1988; Johnson 2002), nor even the first to suggest that restoration practices may be particularly sensitive to interannual variation (Bakker et al. 2003; MacDougall et al. 2008). However, we would like to argue for increased attention on temporal replication in the field of restoration ecology due to its possible sensitivity to treatment by initiation-year interactions.

Many ecologists would agree that the statement "ecology is contingent" may be a fundamental law of our science, yet the effect of the year of initiation on experimental results has been consistently understudied. We found that large majorities of both ecological and agronomic multiple-initiation-year studies reported significant evidence of temporal contingency. This result, coupled with the fact that we found very few ecological studies which incorporated multiple initiation-years into their experimental design, suggests that the influence of this type of interaction has not been fully appreciated in ecology. While the incorporation of temporal replication into the design of ecological experiments may be yet another burden on our limited time and resources, the insights gained from temporal replication may well be worth the effort. We do not wish to argue that all studies initiated in a single year are necessarily shackled by idiosyncrasy, but rather the exploration of this contingency may provide us with a deeper understanding of how ecological systems interact with interannual variability.

Implications for Practice

- Because the initiation year may have considerable impact on the success of a project, bet hedging by planting across years (when possible) may increase likelihood of success.
- Repeating experiments across years may increase the generality and utility of conclusions.
- Temporal replication may also reveal the conditions under which particular treatments are likely to be most effective.

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