Field Studies of Seed Biology
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INTRODUCTION

I like trees because they seem more resigned to the way they have to live than other things do. (Willa Cather “O Pioneers!”)

Except in limited areas where there is enough advance regeneration, establishment of forest cover on harvested lands continues to depend on seedling planting programs or on natural regeneration by seeds. Whereas successful plantation programs depend primarily on plant competition and site variables at the time of planting, successful natural regeneration depends not only on the availability of seeds, but on favourable environmental conditions throughout the processes of seed production, dispersal, germination, and seedling establishment.

Site preparation and other silvicultural treatments can improve the suitability of the seedbed and its micro-environment, but there is still much we do not understand about how various factors contribute to successful forest establishment. We have gained some insights, under controlled conditions, about the influence of major factors such as light and temperature, but we have limited experience with biological responses under actual conditions in the field.

Anyone who has conducted research in the field quickly comes to realize the complexity of the systems chosen for study. An immense number of external and internal factors that affect living organisms must be taken into account—with limited possibilities to control these factors. A major constraint, particularly in a forest environment, is the difficulty inherent in conducting field studies involving seeds. Infrequent seed production, predation by animals, difficulty locating small seeds, estimating the numbers of buried seeds, measuring germination, and monitoring survival pose myriad challenges for the field researcher. Added to these difficulties is the lack of information about effective methods for conducting field studies of tree seeds. A recent assessment of ecosystem management needs stressed the importance of standardized sampling and monitoring techniques, and the lack of consistent methods for archiving, accessing, and updating databases (U.S. Dep. Agric. For. Serv. 1996a). Techniques gleaned from agriculture literature are generally not applicable, and traditional ecological studies (e.g., of seed banks) tend to be primarily descriptive with little emphasis on experimental approaches.

The primary objective of this manual is to detail methods that have been gleaned from the literature and from personal experience of the authors. It is a manual of methods with some general guidelines and interpretation. Relevant background papers are cited where appropriate, but it is not a literature review. The manual is intended for use by researchers in public and private forest resource management agencies, universities, and colleges. Although specifically directed to tree seed research in forested ecosystems, many of the methods described can be used to study seeds of graminoid, herb, and shrub species in both forest and non-forest plant communities. The extensive background information included in the text also provides valuable reference material for many who have an interest in tree seeds, but who are not directly involved in research activities. The detailed examples from previous studies are included, not to prescribe how such studies should be done, but to assist in planning by providing reference values on which to base measurements, sample sizes, and other experimental details.

Since the manual is directed primarily to researchers working in the province of British
Columbia (B.C.), Canada, many examples (forest types, species, research topics), procedures (the biogeoclimatic ecosystem classification system), and regulatory policies are specific to this geographic and political jurisdiction. Nevertheless, it is hoped that the underlying principles are self-evident and will be generally applicable to the conduct of field research elsewhere.
Following a discussion of planning and organizing a field study (Section 1) and setting up an environmental monitoring program for the experimental site (Section 2), the manual is arranged by subject areas most often associated with field studies of tree seeds: natural seed production (Section 3), seed dispersal (Section 4), seed predation (Section 5), seed banks (Section 6), assessing seed quality and viability on emergence (Section 8). Each section was written by one or more experts as follows:

**Carole Leadem**, Ph.D., R.P.Bio., earned her degree in plant physiology from the Botany Department, University of British Columbia, and is a member of the Association of Professional Biologists of British Columbia. She has been in charge of the tree seed biology research program with the B.C. Ministry of Forests in Victoria since 1978. Carole Leadem wrote the sections on planning and organizing field studies (Section 1), natural seed production (Section 3), seed responses to the environment (Section 7.1), seed testing in the laboratory (Section 7.2), seedbed preferences (Section 8.3.1), and contributed to the sections on seed dispersal and silvicultural practices.

**Sharon Gillies**, Ph.D., earned her degree in plant physiology from the Department of Biological Sciences, Simon Fraser University. She has been a biology instructor at the University College Fraser Valley since 1995. Sharon Gillies coordinated compilation of the original manuscript, was responsible for creating the handbook structure and adhering to Ministry of Forests style manual, edited author submissions for the first complete draft, wrote the section on seed dispersal (Section 4), and provided environmental monitoring material for Section 2, and Table 8.1 on seedbed suitability.

**H. Karen Yearsley**, M.Sc., R.P.Bio., earned her graduate degree from the Faculty of Forestry, University of British Columbia, and is a member of the Association of Professional Biologists of British Columbia. Her 15 years of research experience in B.C. include work on ecosystem classification, forest succession, and forest soil seed banks. Karen Yearsley wrote the sections on seed predation (Section 5) and soil seed banks (Section 6), and contributed to the sections on planning field studies (Table 1.1) and seed dispersal (Section 4).

**Vera Sit**, M.Sc., earned her graduate degree from the Statistics Department, Dalhousie University, and is a member of the Statistical Society of Canada. She has been with Biometrics Section, Research Branch, B.C. Ministry of Forests, since 1990. Vera Sit wrote the sections on experimental design and data analysis (Sections 3.6, 3.7, 5.5, 6.4, 6.5, 7.4, 7.5) and the case studies (Section 3.8), and reviewed and contributed to all the statistical sections.

**David Spittlehouse**, Ph.D., P.Ag., earned his graduate degree in forest climatology from the Department of Soil Science, University of British Columbia. His research includes modifying site microclimate to improve seedling regeneration, and determining how forest harvesting and regrowth of the forest affects forest hydrology. He has worked for the B.C. Ministry of Forests in Victoria since 1982. Dave Spittlehouse wrote most of the section on designing an environmental monitoring program (Section 2).

**Philip Burton**, Ph.D., R.P.Bio., earned his degree in plant biology from the University of Illinois at Urbana-Champaign. An independent researcher and consultant, he has been investigating seed biology, forest regeneration, and vegetation dynamics since 1979. Phil Burton contributed material for the
sections on seed dispersal (Section 4), field germination studies (Section 7.3), and effects of silvicultural practices (Section 8).

Each section contains background material on the subject and descriptions of some of the methods and approaches that have been used. There is also advice on experimental design and analysis of the data. Some laboratory procedures have been included to serve as controls for experiments conducted in the field. Laboratory experiments can provide valuable data to supplement field measurements because the results are generally reproducible and environmental variables can be controlled. Many terms are discussed in a comprehensive glossary, and the main subject areas have been indexed.

The logistics of field research are difficult enough in their own right. We hope the information contained in this handbook will help those contemplating new research projects to avoid some of the pitfalls associated with studies in the field. We anticipate other benefits: that this handbook will help standardize field methods and enable comparisons between studies, will increase cooperation between investigators, and will promote more efficient use of resources (equipment, finances, personnel). All of these efforts will help broaden the forest resource database and increase our understanding of the multiplicity of factors involved in forest regeneration.

We anticipate that methods documented in this handbook will be improved once they undergo more extensive field testing, and we invite comments about the information and methods suggested here, and about your own field experiences. Please direct your suggestions to the senior author at the address inside the front cover.
Very special thanks are due to Dr. John Zasada, USDA Forest Service, North Central Experiment Station, Rhinelander, Wisconsin, who reviewed the entire handbook twice. From the time John reviewed the first draft, his interest, enthusiasm, and encouragement have helped immeasurably to sustain our own commitment to the project. His deep understanding of the subject matter emanating from his years of experience in the field has substantially broadened the context and increased the value of the field manual. We thank him, too, for insisting that we include more coverage on hardwood species, forcing us to go beyond the traditional tendency to regard conifers as the only trees in the forest.

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SECTION 1  PLANNING TREE SEED RESEARCH IN THE FIELD

The road to chaos is paved with good assumptions.
(Anon.)

It is much less expensive to learn from other people’s mistakes than your own.
(McRae and Ryan 1996)

1.1 Overview

Forests cover only about 11% of the earth’s surface, yet they account for nearly half of its net primary productivity, and about 70% of the net productivity occurring on land (Whittaker and Likens 1973). Forest ecosystems are complex and diverse, and develop relatively slowly. Thus, forest ecosystem research projects may require years to produce meaningful findings that can be widely applied.

The duration of a study depends entirely on what you want to find out. Many of the biological, physical, and chemical phenomena associated with forest ecosystems can be studied over relatively brief periods on temporary field plots or in the laboratory. To document and compare natural processes, short-term descriptive and baseline studies are essential. Short-term studies also are important to establish the immediate impact of processes on a system, even though they are prone to being confounded by environmental fluctuations such as climate.

On the other hand, many biological phenomena, such as plant succession, occur on time scales of decades or centuries. Long-term studies allow us to evaluate interactions among the various factors controlling ecosystem function that, on a short time scale, might seem inconsequential. Forest ecosystems usually require many years for the effects of perturbations to subside and for long-term trends to appear. This is especially true for communities not in equilibrium, such as those recovering from fire or harvesting.

1.2 General Structure of Successful Field Studies

A survey of long-term forest research programs conducted at many locations throughout the world by Powers and Van Cleve (1991) stressed the importance of planning, commitment, and focus. They concluded that successful long-term experiments shared eight essential components. Not all field studies are conducted over long periods, nonetheless, consideration of the following principles is instructive to anyone contemplating field research, regardless of duration.

1. Sustained commitment
Fluctuations in philosophy, politics, and funding are the surest way to dampen scientific spirit and inspiration. Field studies, once established, must have a fair certainty of continued support, at least to the level required to maintain research sites and to collect core data. This support should be free from political interference. To enlist this level of commitment, researchers should present their arguments based primarily on the benefits that can be derived from investigating socially relevant issues (e.g., sustaining wood production, providing clean water, protecting soils). Proposals that are couched in terms of “understanding how forest ecosystems work” are far less likely to be granted support by funding administrators.

2. Long-term dedication of a site
Plots maintained after the original questions have been answered can continue to have demonstration
value for professionals and the public, and can pay substantial dividends well beyond the life of the original study. Again, the chances of having land dedicated for the site will be enhanced if the research has a central, timeless theme. Support is more likely to continue if research results are disseminated rapidly to administrators and land managers.

3. *A guiding paradigm*
A central focus is necessary to provide structure and maintain research objectives. As long-term objectives may grow hazy with time and personnel changes, periodic reference to the guiding paradigm will help to refocus the research.

4. *A central hypothesis*
A clear statement of the principal scientific question that the research is designed to answer helps to clarify the research direction and stimulate development of the experimental approaches. The central hypothesis is tested through a number of individual studies with definite life spans that terminate once a particular question has been addressed.

5. *Large plots and replication*
Plots should be large enough to simulate natural ecosystem conditions as closely as possible. Large plots not only minimize edge effects, but also increase the flexibility of future studies on research sites. Options might include retaining extra control plots that could later be converted to secondary treatments, or creating split-plots for treatments supplemental to the original design. Large plots facilitate replication of treatments, which is essential for statistical analysis and setting confidence intervals.

6. *Interdisciplinary approach to research*
Field installations should be made available to all research collaborators, regardless of affiliation or specialty. This will attract excellent scientists and promote openness and synergy. Studies that attract a broad array of scientific interests result in much greater understanding than can be achieved through isolated, independent efforts. In addition, program scientists benefit from exchanging ideas, cooperating on experimental work, and collaborating on professional papers. Because collaborators have an interest in continuity and maintaining the research site, interdisciplinary field studies inherently promote the stability of long-term projects. However, interdisciplinary studies only work if there is strong central planning and coordination.

7. *Extension of results*
Research must be designed to make data as portable as possible so that results can be generalized to a variety of species, soils, and forest types. Research will have the highest value if results can be incorporated into a network of coordinated, but geographically separated, studies. Experiments should be sufficiently comparable so that databases can be shared, and each research site should be instrumented so that a baseline of climatological data can be established.

8. *Low red tape*
Maintain the least amount of bureaucratic structure needed to prevent chaos. Initially, a board of senior scientists from a variety of disciplines should review all research proposals. Later, a research coordinator can review projects to ensure that one study does not interfere with another, and that all collaborators are kept abreast of the overall research program. Depending on the size of the research site, a site manager may be needed to facilitate day-to-day (or seasonal) scheduling.

1.3 *Designing a Field Study*
Careful initial planning and organization are critical to the success of any field study. Considering the complexity, the expense, and the duration of many field studies, the consequences of poor planning can be great.

Most studies consist of three stages—planning (Stage I), data gathering (Stage II), and data analysis and interpretation (Stage III). However, the framework for all three stages is constructed during the planning stage. The planning process can be articulated as a series of steps that provide answers to the questions: why, what, how, when, where, how much, and so what.

1.3.1 *Formulating the hypothesis*
The first step before undertaking any field study is to formulate a clear statement of the principal scientific...
question or central hypothesis that the research is
designed to answer—the why of the experiment. A
research plan with a clear statement of the problem
helps to identify the research direction and provide
valuable guidance if the project should run into
difficulty (such as loss of support, changes in per-
sonnel, or environmental disaster).

1.3.2 Stating the objectives
Once the principal scientific problem has been
elucidated, research objectives provide the necessary
structure for planning and executing the project. Ob-
jectives are succinct summary statements of what the
research is trying to achieve. Keeping research objec-
tives in focus during all stages of planning will stimu-
late development of experimental approaches, guide
complete and efficient collection of data, and keep
the research on track.

1.3.3 Selecting the factors to study
The factors to be studied—another aspect of what—are usually specifically identified in the statement
of objectives. The factors chosen will depend upon
whether the study is primarily descriptive or experi-
mental in nature. In experimental studies, factors are
the vehicles through which the objectives are
achieved, and they are generally identified as treat-
ments. Factors may consist of one or several levels.
For example, suppose your objective is to determine
if light affects the survival of lodgepole pine
germinants on open harvested sites. To investigate
this objective experimentally, you would identify light
as the factor to be tested. You might also want to
more specifically compare how different light levels
affect seedling survival; then you would expand the
light treatment to include several levels, such as full
sun, partial shade, and full shade.

Constructing a schematic diagram of the bi-
ological cycle (or other process) is an effective way
to identify what variables affect the process being
investigated. Diagrams help to clarify relationships
and suggest the most appropriate factors to study
(Figure 1.1). For example, if you want to study initia-
tion of reproductive buds, you will want to include
climate, plant condition, and resource availability as
major factors in the experiment. On the other hand,
if you want to examine the factors affecting field
germination, the most suitable variables to study
would be (micro)climate, substrate, and species.

Treatments should be chosen to reflect major
changes in ecosystem function. Viewing ecosystems
under extreme conditions is most likely to reveal
how various ecosystem components function and
to demonstrate the capacity of these components to
recover from change. Studies will have greater value
if the treatments have a generally continuous pattern
(i.e., increasing or decreasing in size). Data obtained
from such treatments lend themselves to predictive
regression analysis. Changes in responses can then be
correlated with changes in the magnitude of specific
factors, and results can be more readily extrapolated
to similar sites. Choosing treatments that span and
extend slightly beyond the full range of expected re-
sponses helps to define the end points and establish
the limits of the system under study.

Particularly in long-term studies, it is advisable
to retain some flexibility in the original design by
incorporating ways the experiment can be changed
if future circumstances should require it (Leigh et al.
1994). To ensure the longevity of the field site, it is
best if only minimum changes are made to the treat-
ments. Changing the experiment to obtain more
information in the short term generally results in
sacrificing the longevity of the treatments. It is some-
times advantageous to incorporate innovations in
forest management practices into the study, but this
should be done only if the major objectives can be
retained. Another possibility is to modify the original
objectives and continue the experiment in a different
form, but again longevity will be lost. A final, but
generally less desirable option, is to set aside the site
indefinitely or reserve it for future use.

1.3.4 Selecting the methods
Methods can be considered the how of experimental
studies. The techniques chosen for the study will
be governed by the study objectives, and the most
effective means of achieving those goals. Usually,
more than one method will achieve a particular
purpose, and for this reason a variety of methods for
field studies of tree seeds has been included in this
handbook. Ultimately, the choice of the most suitable
technique will depend on the available resources.
In most cases, the final decision will be based on
balancing the trade-offs between the detail desired and the constraints of time and money.

Most research projects will employ a variety of methods. Often the distinction between different methods is determined more by the purpose than by the type and intensity of the monitoring. Note that different objectives do not necessarily require distinct and independent data collection efforts. There may be some overlap in the data needs. As long as the research objectives are kept clearly in mind, taking advantage of this overlap can result in substantial cost savings.

Answers to the following questions will help in choosing appropriate methods: What is the primary purpose of the measurement? How well does the chosen method quantify the factor or characterize the intensity of the response? How precise or accurate is the method? How many measurements are required? If many or frequent measurements are required, can the process be automated? The choice may also be affected by the logistics of the experimental site—certain techniques may not be suitable for field use. For example, precise and automated methods may be ideal for making a particular measurement, but the instruments may not be robust enough for field conditions or may require an external source of reliable power.

After choosing the methods, it should be determined whether the research data are continuous or categorical. The term continuous implies that the measurements, in theory, belong to a numerical scale consisting of an infinite number of possible values. However, sometimes you need to measure a quality or condition that cannot be expressed on a continuous scale. This discrete, noncontinuous type of data is called categorical because it generally consists of the number of observations falling into prespecified classifications, groups, or categories (e.g., number of seeds that have or have not germinated).

**Figure 1.1** Framework for evaluating the seed reproduction process in boreal forest trees (adapted from Zasada et al. 1992). A schematic diagram can help to clarify processes and suggest factors for the study.
Continuous data can usually be analyzed using parametric methods such as ANOVA, regression, and MANOVA (Sections 6.5, 7.2.5). However, nonparametric analysis is sometimes required for continuous data if the assumptions of ANOVA, for example, cannot be met. Categorical data, on the other hand, are usually analyzed using methods such as contingency tables and log linear models (Sections 5.5, 7.2.5) although alternative nonparametric techniques are available, if necessary.

Data can also be categorized by the type of measurement used for collection. Determining the type of measurement needed helps to identify the type of information required, the frequency of data collection, and the most suitable means of analysis. Types of measurement include assessment, inventory, monitoring, and visual data.

An assessment is an estimation or evaluation of the significance, importance, or value of a quality or character. It generally implies a subjective judgement (e.g., maturity) to determine placement in a class. The classification scheme may be based on some arbitrary characteristic or a ranked order. Assessment data are usually nonparametric.

An inventory is an itemized list or catalog that may or may not be organized into groups. Usually the number of items in a group are simply counted, with no additional judgement or interpretation. For example, for an inventory of seeds in a seed bank, a count is made of the number of seeds by species present in the soil. An inventory is usually a one-time measurement, but it can be repeated periodically (e.g., annually). Greater use often can be made of inventory data if the samples are stratified in some way, for example, by making separate seed counts at various depths of a soil core, rather than performing a single count of the total core. Depending on the manner in which samples are taken, inventory data can be parametric or nonparametric.

The term monitoring is used to describe a series of observations made over time. The repetition of measurements to detect change over time is the quality that distinguishes monitoring from the related processes of inventory and assessment. The data obtained can be parametric or nonparametric. MacDonald et al. (1991), in compiling guidelines for monitoring water quality, recognized seven types of monitoring: baseline monitoring, trend monitoring, implementation monitoring, effectiveness monitoring, project monitoring, validation monitoring, and compliance monitoring.

Visual data are another significant source of primary scientific information, although they are not generally considered as data. Visual representations may be the most effective way to present information that otherwise would be too unwieldy or difficult to understand (e.g., site maps or structural diagrams). Some information can only be captured visually (e.g., seed X-rays or photomicrographs of plant structure). Although not quantitative, visual data represent an important source of research information, and a valuable means of portraying certain characteristics. Unfortunately, visual data are underutilized in most research studies.

1.3.5 Setting the time frame and determining a schedule
Before starting the study, a schedule should be prepared to outline the temporal distribution of the major components of the study. This is the when of experimental studies. Many field studies are short in duration, but some studies can be very lengthy, such as the ecological studies of the Carnation Creek watershed on Vancouver Island, which have been under way for over 20 years.

The experimental design and type of data analysis will direct how often to collect the data (e.g., daily, weekly, monthly), but the timing of treatments must also be taken into consideration when designing field studies. Treatments may be applied only once, repeated at fixed periods (e.g., annually), or even rotated. The timing will also depend on what type of information is required—whether you are interested in the direct effects in the year the treatment is applied, the residual (or carry-over) effects in subsequent years, or the cumulative effects of repeated treatments.

The length of the study periods should be clearly defined, especially when planning a long-term study. The most suitable period length is defined by how long plot management can be kept constant. Period length might also be governed by the time when the first full assessment can be made (e.g., at the end of the first growing season), or when treatment differences might first be discernible.

In some instances, period length may be used to apportion temporal variation (McRae and Ryan 1996).
In the same way that blocks are used to control spatial variation among plots within a site, changes over time can be partitioned into periods. Although period lengths may sometimes differ because of operational constraints, analysis is simpler when all plots have study periods of the same length.

1.3.6 Choosing the test conditions

The next step is to determine where the factors will be tested. Depending on the research objectives, the test conditions are sometimes considered to be a factor of the experiment. If this is the case, you should choose test sites or conditions that follow some sort of progression or gradient (e.g., small to large openings, low to high elevation). This will allow the results to be more readily generalized to other sites (if the requisite experimental design criteria have been met).

Most details relating to test conditions will be specific to the study and what you are trying to ascertain. For further details refer to the section of interest: seed production (Section 3), dispersal (Section 4), predation (Section 5), germination of seed banks (Section 6), laboratory and field germination tests (Section 7), and silvicultural practices (Section 8).

1.4 Experimental Design

If you don’t deal with each of these levels of variation, your sampled population may not be representative of your target population, and in that case a statistician or a sharp lawyer can make you and your data look pretty lame.

(MacDonald and Stednick 1994)

Once the objectives are identified and the factors, methods, and test conditions are established, attention should be turned to experimental design. The experimental design will prescribe how essential elements of data collection (Stage II) and data analysis and interpretation (Stage III) are executed. Field studies require substantial commitments of time, labour, money, materials, and maintenance; inadequate attention to details such as experimental design and data management can pose considerable risks to the resources invested in the project. Losses due to errors in experimental design may severely damage a scientist’s reputation and will reflect badly on collaborators.

1.4.1 Basic concepts

It is assumed that readers of this handbook have some knowledge of statistics, and will know where to obtain assistance for particular statistical problems. The statistical discussions included in various chapters are intended only to provide general background on important aspects of experimental design and data analysis, and to raise awareness of some potential pitfalls or problems that may be encountered in specific topic areas. Discussions relating to some common statistical methods can be found in the following sections: summary statistics (Sections 4.5.1, 6.5); ANOVA (Sections 3.7, 4.5.2, 6.5, 7.5); regression (Sections 4.5.3, 6.5, 7.4); correlation (Section 3.7); and chi-square (Sections 5.5, 8.3.4).

Careful study of the proposed designs can be invaluable during the planning stage (McRae and Ryan 1996). Trial analyses will demonstrate whether the contrasts of interest can be estimated, and will point out deficiencies in the design and analysis methods. A postmortem of similar experiments often provides data sets and estimates of experimental errors that can be used to evaluate the proposed design. As in the actual experiment, there should be sufficient replication to achieve the degree of precision required to detect the treatment differences. If trial analyses reveal it is unlikely that differences will be found, the study may not warrant the investment. This is especially critical for long-term studies.

The distribution of replicates in space and time is the most critical element of experimental design. Randomization provides for estimates of the experimental errors, which should always be reported, either as the standard error of the mean or the difference between means (McRae and Ryan 1996). Replication is generally accomplished by applying treatments to two or more plots within the site (often divided into blocks) and/or by repetitions of the experiments at other locations or times.

The allocation of replicates will be largely a function of the objectives and the expected variability (MacDonald and Stednick 1994). The more sources of variability you address, the more reliable your results will be. In general, it is not efficient to test for all sources of variability everywhere. However, if you do not repeat any of your measurements, you have an unknown source of error that will weaken all your subsequent conclusions. Repeated measurements
can give a better estimate of a variable such as seed production or field germination, but when you are replicating your measurements, you have to be clear about the level of variability with which you are dealing. Measurement variability is very different from the variability between experimental units (e.g., field germination plots).

Each time you design a project, you need to identify all these potential sources of variation, and determine how you want to deal with them. If you don’t deal with each source of variation, your sampled population may not be representative of your target population. Hurlburt (1984) uses the term pseudoreplication to refer to the testing of treatment effects with an inappropriate error term for the hypotheses being tested. You can think of it as a source of variability which is inherent in the data but cannot be defined because of the sampling strategy. In other words, if you have some sources of error in the data that cannot be tested, then typically you are dealing with pseudoreplication.

One of the examples Hurlburt (1984) uses is a study to determine the effect of water depth on the rate at which leaves rot in a lake. Although this is not a seed-related example, it is worthwhile using here because it is so clear and succinct. Four bags of leaves were placed together at one location at a depth of 1 m, and another four bags were placed together at another location at a depth of 10 m. After some time the bags were retrieved, dried, and weighed. If there was a significant difference between the two sets of bags, all that can be said is that there was some difference between the two locations. To make an inference about the effect of a particular water depth on leaf rotting in this lake, the bags would have to be distributed at the same depth around the lake. To make a more general statement, replicated samples would have to be distributed at different depths in several lakes. The design depends on the question you want to answer, but placing all the bags in one place is pseudoreplication. From Hurlburt’s point of view, you must have replication on at least one level. If you don’t have the ability to test for differences, it is not an experiment.

Often you need to make statistical compromises, and if so, you should be explicit about the statistical trade-offs that you have made, rather than letting them be set by neglect or default (MacDonald and Stednick 1994). For example, in natural resource management, the significance level is typically set at \( \alpha = .05 \), meaning that there is a 1 in 20 chance that an observed difference will be due to chance. A strong level of significance combined with high variability means that usually you will not detect a statistically significant change until damage to the resource has occurred. However, given the high natural variability in natural systems, it may be better to use a less stringent significance level in exchange for a higher level of resource protection. Another example is power, which is usually designated as 1-\( \beta \). When comparing two sample means, the quantity \( \beta \) is known as a Type II error, which is the probability of incorrectly concluding that two populations are the same when in fact they are different. Again, if a resource is slow to recover or is of high value, you probably want to increase the value of \( \alpha \). In natural resources management \( \alpha \) should probably be set at .10 (MacDonald and Stednick 1994).

Excellent discussions of pseudoreplication can be found in Stewart-Oaten et al. (1986), Bergerud (1988), and MacDonald and Stednick (1994). Additional discussion of randomization and replication in relation to field studies of tree seeds can be found in Section 7.4.3.

Blocking of the plots reduces experimental error by removing any gradient effects in site variation. Choosing blocks that are arranged contrary to the field gradient will increase the experimental error, but this is difficult to avoid because the field gradient is often unknown. Appropriate variables on the site (temperature, soil, moisture) can be measured and the results used as a basis for blocking. If elevations of the site vary considerably, blocking would most likely be parallel to contour lines, and not perpendicular. The effectiveness of a block arrangement can be assessed only after the experiment has been run. A good strategy is to have a robust blocking structure that allows for an environmental gradient in either or both directions in a rectangular site layout (McRae and Ryan 1996). See also Section 7.4.

In forestry research the same unit or process is usually measured on more than one occasion. For example, in trials to compare several treatments, data are typically collected before and after treatments are applied. Such data tend to be serially correlated, or autocorrelated, which means that the
most recent measurements are dependent on, or to some extent predictable from, earlier observations. Because this violates the independence assumption on which many standard statistical methods are based, alternative methods are required for their analysis. Two broad classes of methods have been developed for this purpose: repeated-measures analysis and time-series analysis. For additional background and discussion of this topic, refer to Nemec (1996).

Carry-over effects from previous treatments are a hazard of long-term studies in which multiple treatments are applied. When analyzing by ANOVA or multiple linear regression, estimates of the direct effects of later treatments must be adjusted for any residual effects remaining from previous treatments (McRae and Ryan 1996).

1.4.2 Determining the sample size
To ensure that enough samples are collected for a study—the how much of the experiment—it is advisable to determine the appropriate sample size specific to the parameter being studied.

Sample sizes for each measurement must be determined independently, because variability may be different for different characteristics. For example, the sample size required for measuring cone characteristics may not be the same as that needed for seed characteristics, even for the same species, because of differences in the variability of the data (Carlson and Theroux 1993). Environmental changes may also result in year-to-year variations, but these differences can sometimes be minimized by adjusting all values to be relative to those observed in a particular year (Ager and Stettler 1983).

Sample size is usually determined by applying statistical efficiency calculations to a preliminary set of measurements (Sokal and Rohlf 1981; Ager and Stettler 1983). See also Stauffer (1981, 1982) for sample-size tables oriented to forestry applications. In the absence of any other information, a sample size of 10 is often a good place to start (MacDonald et al. 1991; MacDonald and Stednick 1994).

The topic of sampling, both how to sample and how many samples to collect, is a critical aspect of all field studies of tree seeds. For more detailed discussions relating to particular subject areas, see Section 3 for seed production, Section 4 for seed dispersal, Section 6 for seed banks, and Section 7 for seed germination tests. A more general discussion of various types of sampling can be found in Cochran (1977) or Thompson (1992).

1.5 Data Management
Data management protocols should be established when the study is initiated. The type of data, experimental design, and method of analysis will guide how the records and data are organized and managed. This section provides a brief overview of the major points of data management, as well as some special considerations required for long-term studies.

1.5.1 Establishing a coding scheme
A consistent coding scheme should be established to correlate all data records with the research plots and treatments. The coding scheme is best defined in a table assigning unique label codes to identify field plots, factor levels, treatments, and replications. The table should indicate the exact units in which the data will be recorded (e.g., millimetres, kilograms, or watts per square metre). For categorical data (Section 1.3.4), a brief description should be given of the significance of each classification code (e.g., in Section 3.5.3, for cones, 1 = scales fully open; 2 = scales partly open; 3 = scales completely closed).

Allow for some flexibility in the coding scheme so that labels can be added if new treatments are incorporated, or if treatments change over time. If treatments are changed, ensure that the coding scheme is annotated to relate the new treatments to the original treatments.

The same format should be established for field and computer records so that data can be easily accessed for future examination or analysis. Where feasible, coordinate with other agencies or researchers to use standard codes or data-entry protocols. This will facilitate exchange of data between programs. For example, the standard coding formats used for the biogeclimatic ecosystem classification data should be used for all site and vegetation data. Standard species names and codes for British Columbia can be found in both Access 2.0 and Excel 4.0 files at the B.C. Ministry of Forests Research Branch FTP site (see Appendix C) in the directory/pub/provspp. The files are regularly revised and updated. If you want
to collect vegetation data, then you should follow *Describing Terrestrial and Aquatic Ecosystems in the Field* (in preparation 1997) which will update Luttmerding et al. (1990). This is also a useful reference for making site descriptions (see Section 1.6.5). A variety of computer data entry and reporting tools (e.g., *Venus*) are also available (see Appendix C for more complete information).

### 1.5.2 Creating a permanent file
The permanent file should include the initial plans and objectives and all parameters of the experiment. A statistical guide should be included in the permanent file giving full details of the experimental design(s), the proposed method of analysis, and all associated computer programs. Include the type and number of annual data sets, and a list of the different annual records. Special notes about the trial should be recorded and arranged by date or other logical sequence. Include maps giving details of the research sites, the location of the plots, and the arrangement of the treatment blocks and replications (see Figure 1.2).

![Figure 1.2](image)

*Maps giving details of the research site should be included in the permanent file. (a) Locations where western larch and subalpine larch are sympatric (Carlson and Theroux 1993). (b) Sketch of investigated stands of Pinus sylvestris (Bergsten 1985).*

Provide room in the structure of the permanent file so that you can add data and field notes for the current year and update the parameters, if necessary. It is useful to link computer data files to a spreadsheet or graphics program to produce a series of graphs depicting the different responses over time for each treatment. Create a summary table of the cumulative effects for each variate, giving the relevant summary statistics.

Plan the permanent file so that computer formats and files will remain compatible over changes in computers and software. To ensure efficient data entry, carefully design data sheets and format computer files. The spreadsheet software into which you plan to import your data should guide the data file format. For most data, a row/column format is best. If you are uncertain about the type of software that will be used, a simple **ASCII** (text) file format is recommended.

The permanent file should also contain detailed directions for finding the plots again after installation. The importance of this step cannot be emphasized enough, especially if different people are resampling the plots. Several scales of maps are needed to relocate plots. Section 1.6.4 includes a more detailed discussion of recording site parameters for relocation.
1.5.3 Preparing to collect data and samples

Computer-generated administrative aids (labels, data sheets, random order lists) will simplify data and sample collection. Organic tissue and soil samples collected during the study must be properly coded and archived for analysis or future reference. Pre-printed labels simplify collection of samples in the field and act as an additional check that coding sequences are complete. Colours and symbols (e.g., stars, circles, triangles) used in addition to, or instead of, numerical codes will help to reduce errors, which may result from performing repetitious tasks under arduous field conditions.

The sequence prescribed by the randomization scheme can be used to arrange labels, sample containers, and data sheets. If you have a large number of items, it may be convenient to subdivide them into smaller groups (e.g., by plot number).

Permanent markers (stamped metal or plastic are best) should be generated for all treatments, and securely attached to durable, highly visible posts or other stationary devices in the field plots. For further details, see Section 1.6.4, Table 1.1.

1.5.4 Collecting the samples and recording the data

Data can be recorded in the field using manual records or hand-held dataloggers or other automatic recording apparatus. Pre-labelled sheets can be used for manual data entry, or datalogger files can be pre-programmed with plot, treatment, and sample codes. Refer to Spittlehouse (1989) and Section 2.2 for additional information on using dataloggers in the field.

Transfer of data is now relatively easy using computers and computer interface devices, but all files must be regularly backed up to avoid loss of data. You should have spare power sources, in case primary equipment fails.

1.5.5 Reporting

A complete analysis of the research and a summary report should be prepared annually or at the end of each field season. This can be considered the so what of the experiment—what do the results mean in the greater scheme of things. Strive to disseminate as quickly as possible the interim results or updates at technical meetings, in short articles, or in newsletters. Prompt reporting will help maintain support while the research is in progress.

1.6 Selecting and Describing the Study Site

1.6.1 Selecting the study site

An essential part of planning is selecting a suitable site—the where of the experiment. Site selection should take into consideration practical aspects such as accessibility, the frequency of site visits, and how seasonal changes may affect access and any permanently installed instrumentation.

As early as possible in the planning process, contact the local forest district or the forest management office responsible for the proposed study area. Establishing a good relationship with those ultimately responsible for the site can have unexpected benefits and will also serve to promote your research amongst the forestry community. Local staff may be able to suggest potential sites that meet your criteria and provide more detailed information if they know the objectives and the key factors you wish to study. From them you can gain considerable information and knowledge about local forestry practices that will directly and indirectly influence your work or research, now and in the future. Because they are located near the site, they may be able to maintain security or assist with site maintenance. In addition, if local forestry staff know about your study, the chances of the trial being damaged by concurrent industrial or silvicultural activities is greatly reduced.

The scientific criteria for selecting a site depend on the goal of the study, but all critical site-related factors must be identified. For example, if the objective of the study is to determine the difference in seed production between north- and south-facing slopes, then site selection will be dictated by the aspect and grade of the slope. In other studies, slope and aspect would not be primary factors. If the goal is to describe seed production in mixed stands as opposed to uniform stands, then the primary selection criteria would be the species composition of the stands. For long-term research on seed production in a natural stand, it would be important to locate each site away from openings or roads. In this case, a fixed area on each site might be delineated in the centre of the stand, with the trees surrounding the plot acting as a buffer zone to reduce edge effects.

Site illustrations are useful in documenting the key elements of the field site (Figure 1.2), and should form part of the permanent file (Section 1.5.2). They
may also be used to find the plots again for repeated measurements (Section 1.6.4).

1.6.2 Deciding on temporary or permanent plots
A decision must be made whether to use temporary sites (entirely new units are randomly selected for observation each time), or permanent sites (the same units are observed repeatedly over time). The choice of temporary or permanent plots depends on the degree of correlation you expect between the initial and final plot values. If a high positive correlation is desired, permanent plots will generally give better precision. If large-scale changes are expected in the nature of the site, temporary plots should be used (Freese 1962). Sometimes a combination of temporary and permanent sites can be used—permanent (intensive) sites for detailed aspects of the study and temporary (extensive) sites for broadening the number of samples and site types.

1.6.3 Determining size and shape of the plots
The size and shape of the plot depend on a number of factors, including the goal of the research, the cost or time required for sampling, the required precision, and the uniformity or heterogeneity of the area (Freese 1962). There are obvious trade-offs between plot size and homogeneity of samples. You want as large a sample size as possible, with good treated buffers, but the larger the plot size, the more likely you are to introduce heterogeneity (in soils, nutrient regime, moisture regime, slope, etc.) into your plot selection, thereby increasing the within-plot error sources. To assess homogeneity, a full site description of each plot is recommended. Plots should only be accepted for inclusion in the study if the variation in site type would not compromise the long-term results. In general, moving more than one full site series (or other environmental gradient) within a plot is probably sufficient reason to abandon it. Larger plots (more than 40 × 40 m) should have multiple soil pits to ensure homogeneity.

The duration of the study will also govern plot size. If there is any possibility of continuing the study for 5 years or more, consider the plot size carefully. For example, if your original objective was to study germination and initial development, but later you decide to extend the length of the study, you may be unable to do so if the plot size initially chosen was too small.

The choice of plot size often depends on stand density and heterogeneity. To compensate for differing stand densities or species mixes in a study, the plot size could vary to maintain a constant number of trees or species types within each plot. See Smith et al. (1988) for an example of this approach.

Another approach for studying tree density effects is to use rectangles of fixed dimensions (width and length) for all sample areas. Tree density can be estimated by dividing the number of healthy trees within a sample rectangle by the area of the rectangle. This approach is preferable because the fixed dimensions provide consistent estimates of site variation across all study areas, and ensure the validity of tests for tree density effects.

1.6.4 Installing, marking, and relocating the plots
Once the experimental site has been selected, the plots must be identified and the boundaries clearly marked. Markings must be highly visible and durable. The choice of marking method will depend on a number of site factors such as the distance from the road, steep terrain, annual snowpack, rocky ground, or height of vegetation. The durability required of markers will depend on the amount of exposure to the elements, the possibility of crushing or toppling by large animals (bears, moose, cows, humans), and the total length of time the plot will be sampled. A summary of important points for installing and marking plots is given in Table 1.1.

After installation, the site location should be recorded in detail in the permanent file. This is an important step, and will prove particularly valuable if the plots are resampled by different people or over many years.

- **To locate the general vicinity of the site:** Mark site locations on topographic maps, forest cover maps, airphotos, orthophotos, etc. Write out directions including distances (km) to each turning point and road names, etc., from likely starting points (towns). Use GPS locations if you can afford and have access to this technology.

- **To locate the site from the point of access (e.g., road):** Draw a site map of the plot(s) and surrounding area with local landmarks (e.g., roads, water, rocks, slopes, directions, etc.). If there is more than one plot, ensure
they are mapped in relation to each other as accurately as possible, using compass bearings and distance measurements.

- **To locate the plot(s):** Flagging tape and painted stakes will help you spot the plots once you are at the site (See Table 1.1). Plots marked with metal stakes, pins, or tags can be relocated with a metal detector.

- **Take photographs of the plot** to have a visual record of changes that occur and assist in relocating the plots. Mark the photo points on your site map.

### 1.6.5 Describing the site

General site characteristics should be described for a field site even though they may not be identified as the primary factors under investigation. A site description should include the slope, aspect, elevation, longitude and latitude, soil classification (Agriculture Canada Expert Committee on Soil Survey 1987), humus classification (Green et al. 1993), and for sites in British Columbia and some other areas, the appropriate biogeoclimatic ecosystem classification (refer to the B.C. Ministry of Forests regional field guides listed in Appendix C). Topographic grid references are also useful to locate the general area of the site.

Keeping the objectives of the study clearly in focus will help identify other factors that should be documented in the site description because they might affect the outcome of the study. For example, the percent cover of major non-tree species that commonly invade sites following disturbance should be listed if their presence could influence the results of your experiment. Soil profile details could be included if the experiment would benefit from this information. If the site has been harvested, the degree of soil disturbance should be quantified and

**TABLE 1.1 Installing and marking the research plots**

<table>
<thead>
<tr>
<th>Stakes</th>
<th>Weight:</th>
<th>Visibility:</th>
<th>Wood:</th>
<th>Steel reinforcing bar (rebar):</th>
<th>Aluminum:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>This factor is critical (unless few stakes are needed) if the site is inaccessible and materials have to be carried a long way. In rocky ground, thinner stakes are easier to install. On steep slopes, stakes usually get pushed over in the winter, especially where there is a lot of snow and/or vegetation. Use strong, slim stakes and pound them far into the ground to reduce this problem.</td>
<td>Stakes should be taller than the tallest understorey vegetation, but short enough that you can reach the top to pound it in. Allow enough length to compensate for the amount that is pounded into the ground. Ensure the stakes are clearly visible by painting the tops bright, contrasting colours (white, fluorescent pink, orange, or blue; not yellow, green, or dull or dark colours).</td>
<td>Pros: relatively lightweight; broader surface more visible when painted; easy to attach labels; moderate cost. Cons: bulky; eventually rot; can split and break; may be harder to pound into the ground; greater surface area, more easily pushed over by snow.</td>
<td>Bend the ends of rebar stakes to prevent injury to people and animals. Pros: compact, long-lasting (but rust); relatively cheap; easy to pound into even rocky ground; can be relocated with a metal detector. Cons: heavy; not very visible even when painted, difficult when rusty; harder to attach labels; &lt;1 cm diameter can bend fairly easily; larger diameters (&gt;1 cm) are too heavy (except for short stakes).</td>
<td>Use either conduit or Y-beam. Pros: lightweight; visible when unpainted; strong (doesn’t bend easily), won’t corrode, can engrave plot information directly on so don’t have to attach separate labels; can be relocated with a metal detector. Cons: expensive (3–4 times cost of rebar).</td>
</tr>
</tbody>
</table>
**TABLE 1.1 Continued**

**Installation**

Pound stakes until they are as steady as possible. Carpenters’ hammers (unless they have metal handles) break too easily. A short-handled 2 lb. (0.9 kg) sledge hammer is ideal because the handle is stronger and the head has a broader surface area. If toafil (hipchain) is used to measure distances, remove the thread after measurement because it can entrap birds and other small animals and kill them.

To form square plots: Use one rope to measure the length of two sides of the plot (marking the middle), and a second rope for the diagonal length. A tape measure can be used instead if it is long enough. Install the first stake. Measure the distance to the diagonally opposite stake and install. Measure the length of a side towards a third corner from each of the first two stakes. Where these meet, install a third stake. Repeat the last two steps to locate the fourth stake. Ropes with loops on the ends (one the diagonal length and the other the length of two sides with the middle marked) or two flexible fibreglass tapes can be used to make the measurements.

For circular plots: Install a single stake in the middle of the plot. Use a rope the length of the plot radius to measure from the centre stake to the plot boundaries and mark with flagging tape.

**Labelling**

Labels are essential if there is more than one plot in the installation. Identify the plot with a number code and other pertinent information. (See also Section 1.5.1.)

- **Washers:** Stamp large washers (3.5 cm diameter with 1.5 cm hole) with plot numbers using a die set, then slip them over the rebar stakes. **Pros:** easy to use. **Cons:** eventually rust so much you cannot read the numbers; work their way into the ground and must be excavated; can slip off and be lost if the stake is pushed over in the winter.

- **Aluminum sheets:** Can be wired, nailed, or stapled onto wooden stakes, or folded around rebar stakes and stapled. **Pros:** easy to engrave; won’t corrode; easy to see; can attach them to the tops of stakes (no excavating); easy to mold to stake. **Cons:** easily ripped by animals or vegetation rubbing in winter, etc.; sharp edges unless each edge is bent.

- **Plastic or metal tags:** Can be purchased from engineering or survey equipment suppliers either pre-numbered or blank. Some suppliers will engrave custom numbers on blanks. Pre-numbered plastic livestock ear tags are also available from agricultural suppliers. Plastic tags come in different colours but may break or fade over time. Metal tags are more durable but less visible. Use coated wire to attach tags to plot stakes, trees, etc.

- **Flagging tape:** If resampling frequently (e.g., every 1 or 2 years or less), use plastic flagging tape on stakes. Use the most durable winter-weight flagging; although more expensive, it lasts much longer. Use fluorescent pink, orange, etc. (same as for paint) for the best visibility. A long tail of tape moving in the wind will catch the eye better than many short pieces and wrap-arounds. Biodegradable tape is not recommended as it is almost impossible to see. If sampling is infrequent, don’t bother with flagging; it is not very durable, and animals chew on it. Rely on painted stakes, photos, and good site maps instead. Felt pen on flagging tape is OK for temporary labelling purposes (about one year), but not for long term.

When a stand is present on the site, then the description should include an estimate of tree species composition based on basal area. This can be done using variable-radius sample plots. The basal area factor of the prism or relaskop and species of “in” trees are used to estimate species-specific basal area (British Columbia Ministry of Forests 1992). Relaskops are used most commonly, but a set of prisms works as well and is less expensive (about $40 instead of $1400 for a relaskop).

Environmental conditions, such as relative humidity, rainfall, hourly temperature averages, and daily maximum and minimum temperatures, can be monitored using on-site dataloggers. Note that climatic data collected from standard weather stations may not be sufficient to accurately document factors that affect flowering, pollen dispersal, cone opening, and seed maturity at the stand level. Weather variables monitored several metres above the ground may not reflect the conditions within the crown, or on the north and south sides of a tree. In some cases it may be useful to establish correlations to capture these relationships. For further discussion on these and related topics, refer to Section 1.5, Data management, and Section 2.2, Designing an environmental monitoring program.

1.6.6 Site index
The site index is commonly used in forestry to measure site productivity. The site index is the average height of top height trees (unsuppressed dominant or codominant trees) measured at breast height age 50. The more productive the site, the higher the site index. Site index can be obtained in at least two ways: from tree measurements or from the site series. To obtain accurate tree-based estimates of site index, good top height trees should be present in the plot. A description of what constitutes good top height trees can be found in Forest Productivity Councils of British Columbia (1996) and Soderberg and Nigh (1994). These publications also detail the sampling protocol. The total height of the tree and its breast height age are required to estimate site index. This information and the name of the tree species can be entered into the SiteTools software (available from Research Branch, B.C. Ministry of Forests) to obtain an estimate of site index.

When good top height trees are not present on the site, the site index can be approximated through a site series–site index correlation, by which a site index is estimated from the site series present. This method is generally less accurate than the tree-based estimates, and should not be used if good top height trees can be found. Site series–site index correlations are not yet widely available, but may be found in the Ministry of Forests field guides for Nelson (Braumandl and Curran 1992), Prince Rupert (Banner et al. 1993), and Vancouver (Green and Klinka 1994), and in the scientific literature (Green et al. 1989; Klinka and Carter 1990; Carter and Klinka 1992; Wang et al. 1992; Kayahara et al. 1993; Wang et al. 1994). First approximation provincial correlations are available in draft form (Meidinger and Martin [1997]).

1.7 Analyzing and Interpreting the Data

The great tragedy of Science: the slaying of a beautiful hypothesis by an ugly fact.
(T.H. Huxley)

For many researchers, the most enjoyable (and challenging) part of a study occurs after the data have been acquired and entered into the database. At the data analysis and interpretation stage (Stage III) the relevance of research results must be recognized and articulated. If the project has been well planned (including site selection, experimental design, and analyses), this stage is usually straightforward. However, unexpected things happen, and you may need to manipulate and analyze the data in ways not initially planned. For example, look for confounding factors that may be influencing your results, or try grouping your data differently and reanalyzing.

The value of reanalysis is best demonstrated using an example (D. Coopersmith, pers. comm., 1997). It also demonstrates how a detailed site description can later be used for other purposes.

Recently, an analysis was performed on permanent sample plots at the Aleza Lake Research Forest in north-central British Columbia (lat 54°06′N, long
The stand had been logged in the 1940s using diameter-limit selection (all trees larger than 40 cm were taken, and smaller trees were left). The site was a very productive moisture-receiving site at the base of a long slope. It was also micromounded, probably from previous windfall events in the stand. Some of the spruce were as old as 200 years, so it was probably 300 or more years since this site had been burned. An initial examination of the tree data showed that basal area and volumes had not increased since the last evaluation in 1986. This was surprising because some very large spruce and subalpine fir appeared to be growing very vigorously on the site.

A second analysis was performed. This time the trees were separated into two classes: those growing in the wetter hollows of the micromounds (characterized by *Equisetum*); and those growing on the drier mounds of the site. The results of this reanalysis were dramatic: all trees in the hollows showed little or no growth (in fact, large numbers had died since the last evaluation), showing that trees on these microsites had not contributed anything to basal area and volume in the stand, while those on the higher microsites were still growing vigorously and adding significant additional growth. By not differentiating between these two microsite types, much of the story of these plots was lost in the “noise.”

Researchers should consult a statistician before embarking on any of the more elaborate statistical analysis methods to ensure the proper application of the techniques.

### 1.8 Administration of the Research Site

Field research must always be undertaken with the knowledge and approval of the land owner and the local land manager. In British Columbia, researchers wishing to locate research sites on provincial Crown land must follow the regulations and guidelines set by the B.C. Ministry of Forests and other agencies. The steps outlined in this section are specific for research sites in British Columbia, but are similar to requirements in other areas. Ensure that you contact the agencies with jurisdiction over the area you have chosen and that you know and follow the appropriate regulations.

#### 1.8.1 Obtaining site approvals

For sites in British Columbia, researchers are responsible for obtaining the B.C. Ministry of Forests district manager’s agreement for the location and purpose of the research. The district manager will want to ensure that the project can be accommodated within the objectives of the management plan for the site. Researchers must adhere to the Forest Practices Code of British Columbia; for silvicultural system and natural regeneration trials, this may require amendment of silviculture plans and cutting permits. Plans normally must be filed at least 1 year in advance with the district office. For trials not affecting silviculture prescriptions, the district manager must be notified a minimum of 3 months before the proposed research work.

Before beginning any study on developed lands, check with the local offices of major utilities to ensure that site activities will not disrupt water, electrical, or gas services in the area. Field personnel should know the name and telephone number of the appropriate utility companies to contact in case of emergency.

#### 1.8.2 Registering field installations

Some regional offices maintain a list of the objectives and locations of all known research plots within the region. Researchers establishing research plots or installations on provincial Crown land must convey the site location and other pertinent information to the regional research manager, as well as to the relevant district managers.

A protocol for plot registration and map notation has now been established for all permanent sample plots (*pSp*) by the B.C. Ministry of Forests for the forest inventory mapping system (*famap*). Map notations are made on all mylars furnished to the forest districts. When a district is proposing a stand treatment, such as thinning or fertilization, all district mylars are checked for the area of the proposed treatment. This is how the forest district avoids treating well sites, archaeological sites, and research plots.

There is a standard procedure for getting information entered on the map mylars, such as a harvesting tenure or an experimental project (*ep*), usually by providing a sketch map and documentation. Note, however, that the researcher is responsible for keeping track of sub-*eps* in the permanent research file.
1.8.3 Security
For security purposes, the location of research sites must be on file with the applicable district, regional, and licensee offices. Installations that will be repeatedly visited (i.e., more than once) should be registered on forest cover maps as either a map notation (coded on the forest cover map to notify users that an activity is occurring there) or a map reserve (to reserve the site from harvest within a specified time period). Map notations or reserves can be critical in saving a site from disturbance or inadvertent damage.

Experimental plots benefit greatly from having signs posted on the site. This will keep out most of the public. At the minimum, the sign should state “Research Site, Do Not Disturb. If you would like more information, please contact the nearest district office.” Locate valuable equipment such as meteorological stations so they are not visible from the road; this will lessen the possibility of equipment being vandalized or used for target practice.

1.8.4 Safety
Ensure that you know the radio and check-in protocols for the district you are working in (see B.C. Ministry of Forests Research Branch Operating Policies and Procedures).

Use radio or cellphone communication when possible. Radios are essential on active logging roads. The forest district office or logging company can supply you with radio frequencies, but they are also usually posted at the beginning of the road. The appropriate frequencies can be programmed into radios by staff of the B.C. Ministry of Forests Technical and Administrative Services Branch (for ministry employees) or at most radio shops. In the field, call your location frequently and monitor the location of logging trucks so you can pull over well before you meet them. Logging trucks always have the right-of-way. Always drive with your headlights on when on logging roads.

Report your destination and return time before any field trip, and check in during the day so that someone knows where you are and your next stop. B.C. Ministry of Forests policy advises against working alone in the field and strongly discourages the practice. This policy relates specifically to situations in which an employee is working in a remote area off paved roads, and may be unable to call for help if injured, or is unlikely to be discovered by passers-by. If working alone is necessary, you should know and closely adhere to policy guidelines of the forest management agency, your employer, and your local authority.

In British Columbia, the safety of crews working in the field is governed by Workers’ Compensation Board (wcb) regulations and Industrial Health and Safety and Occupational First Aid Regulations. Ensure that you are aware of and comply with all the requirements—only a few are highlighted here. Regulations stipulate that a Level I first aid kit and someone with appropriate first aid training must be on-site. Specific written procedures for transporting injured workers must be developed and be present at the field site before operations begin.

1.8.5 Using registered seeds
In British Columbia, only registered seeds may be used for reforestation on Crown land (Forest Practices Code, Silviculture Practices Regulation, Sec. 2(1)(a)). This regulation also applies to forestry research trials if the seeds will be planted on Crown land. Refer to the Forest Practices Code Guidebook: Seed and Vegetative Material or consult with district staff for current seed use guidelines. The Seedling Planning and Registry (spar) system can identify available registered seed sources. Contact the district office for assistance with spar and other Code-related matters. (See Appendix C for resources.)

1.8.6 Making seed collections
If the seedlings resulting from individual seed collections will not be planted on Crown land, the use of registered seeds is not required. However, if you plan to collect your own seeds, you must obtain a cone collection permit from the district office in which the collections will be made. B.C. Ministry of Forests researchers are not required to have a permit, but they are still encouraged to inform district staff of their intention to collect cones.

Anyone making seed collections is responsible for rigorously adhering to all precautions restricting the use of climbing gear and collection equipment. In British Columbia, aerial operations are subject to wcb regulations, the helicopter company must be certified, and the pilots appropriately qualified for making aerial collections. Refer to wcb regulations, Eremko et al. (1989), and Camenzind (1990) for
additional information on the safety aspects of tree seed collection in British Columbia.

1.9 Ecosystem Management

From time to time, it is beneficial for researchers to stand back and view their research in the larger context in which studies are conducted. By viewing studies of tree seed biology in the broader perspective of ecosystem management needs, research results can have a greater impact and enhanced value to society.

Ecosystem management is a scientifically based land and resource management system that integrates ecological capabilities with social values and economic relations to help sustain ecosystem integrity and use over the long term. In recent years, the term adaptive management has been used to describe a modified approach to managing ecosystems. One of the main distinctions of adaptive management is that it emphasizes learning through conscious experimentation, monitoring, and adjustment (U.S. Dep. Agric. For. Serv. 1996b).

The goal of adaptive management is to create and maintain sustainable ecosystems. To achieve the goal of sustainability requires that we integrate both the human societal and economic needs and ecological processes. This concept may be visualized by viewing the needs of society and the earth’s ecological capacity as separate spheres (Figure 1.3). Knowledge and understanding draw these circles closer together. Opportunities for sustainability increase when we manage so that these spheres can overlap.

Information is a primary resource, and as researchers, it is our major contribution; the success of adaptive ecosystem management depends on the generation and transfer of our scientific knowledge (Bormann, Brookes, et al. 1994). Monitoring and research must be integrated with decision-making processes to continually improve the scientific basis of ecosystem management (Jensen and Everett 1994). Thus, it is critical that we allocate our efforts to bridge this interface between science and management. In a topical article, Larsen et al. (1997) defined 10 principles of ecosystem management which provide useful guidance to ecosystem researchers to make their research projects relevant to management needs for information. Not all research projects will be able to strictly adhere to these principles, but they provide a useful reminder of context for natural resource studies.

1. Management and research must deal with large landscapes. The cumulative effects of processes that typically function at smaller scales, such as stand-level silvicultural treatments, can be observed only if we step back to take a wide-angle view of the forest. Some important processes, such as patterns of forest distribution or natural disturbance, can be observed only at the landscape scale.

![Figure 1.3](image-url) **Figure 1.3** Sustainability can only be achieved when the needs of society and the potential capacity of the earth we live in overlap. Learning draws these circles closer together and increases our opportunities and options for sustainability. (Adapted from Bormann, Cunningham, et al. 1994.)
2. **We must be concerned with long time frames.** Just as the extent, structure, and condition of today's forests have been determined by harvesting practices that took place a century ago, so the impact of the current management activities will persist at least a century into the future.

3. **We must consider both where and when we create a disturbance.** Important spatial and temporal components are associated with any forest management activity or any natural disturbance. If, for example, our management activities will disturb large areas in a given landscape over the next century, it makes a difference whether the affected areas are contiguous or dispersed, and whether the disturbance occurs in a single year or is spread over the full century.

4. **We have enough scientific knowledge to start managing ecosystems,** but we will never fully understand all aspects of forest ecosystems. We know a great deal about some parts of forest ecosystems and at least a little about most parts; a prudent approach is to begin by using the best science we have available now, while we continue with our research.

5. **We must synthesize the results of research that address many different ecosystem attributes and processes.** We must combine what we know about ecosystem components and ecosystem processes to arrive at a more complete understanding of how ecosystems work and how they respond to disturbance. Synthesis also serves to identify the major gaps in our knowledge.

6. **The complexity associated with ecosystem management is so great that we must employ mathematical models.** Tracking details, measuring interactions and trade-offs, dealing with long time frames, dealing simultaneously with many species, and mapping the results—all require the use of computer models.

7. **We must facilitate cooperation and collaboration.** The complexity of forest ecosystems requires the attention of teams of scientists and managers representing a wide range of expertise.

8. **Researchers must share sites so that they can integrate their findings and investigate change in each ecosystem component over many different spatial and temporal scales.** Agencies must make long-term commitments to maintain research sites as well as to fund basic site measurements. The marginal cost of additional projects is quite low as long as a base level of measurements exists.

9. **We must simultaneously focus our collaborative research efforts on real landscapes.** We will increase our understanding of the interactions and trade-offs only when experts from many fields apply their collective wisdom to the same piece of land over the same time frame. Purely theoretical approaches to ecosystem management research have great merit, but ultimately the evaluation must be in the field.

10. **We must remember that people are part of the ecosystem.** Human activity has left an indelible mark on our forest resources, and ultimately, it is people who decide which forest practices are acceptable. Our role as scientists and practitioners must be to: (a) identify and discourage those activities that will likely cause short-term or long-term ecosystem degradation, (b) clarify the trade-offs among many acceptable management alternatives, and (c) identify and encourage the alternatives that will most likely produce the desired outcomes.

1.10 **Summary**

*A long-term experiment whose sole sponsor has left, died, or lost interest is a sad orphan, and adoption is seldom quite successful.*

(Dyke 1988)

Planning constitutes the major activity associated with field studies, and may even take longer than the study itself. Care is needed in defining the experimental protocol, data management, and reporting routine. Good plans are especially critical when the main investigators are not readily available at all times during plot establishment and site selection.
Flexibility is also required, and possible modifications should be considered even while the study is being conceptualized. The plan must try to anticipate some level of uncertainty and be flexible enough to cover unexpected conditions in the field. Change is inevitable, and consideration of alternative approaches during the design stage will help to focus the planning effort and secure long-term success of the project.

Field experiments require sustained commitment by the scientific staff so that the study will reach its full potential. Sustained commitment by funding organizations is also essential to maintain stability. Finally, the information needs urgently required by natural resource managers necessitates that the results of field studies reach the end user as quickly and as accurately as possible.

The role of a scientist in the ecosystem management model is to provide information for the decision-making process. Such information helps to identify the current status of an ecosystem as well as potential options for addressing the social, physical, economic, and biological issues (Haynes et al. [technical editors] 1996). This information helps clarify feasible limits, options within the limits, consequences of those options, and trade-offs between options. It is the role of the decision-maker to choose among options; it is not the role of science. The challenge for resource managers is to balance biological science with social science and with the philosophical views of how society values renewable and nonrenewable natural resources (Haynes et al. [technical editors] 1996).
SECTION 2 DESIGNING AN ENVIRONMENTAL MONITORING PROGRAM

Every raincloud, however fleeting, leaves its mark, not only on trees and flowers whose pulses are quickened, and on the replenished streams and lakes, but also on the rocks are its marks engraved …
(John Muir “Gentle Wilderness, the Sierra Nevada”)

2.1 Background

Environmental and site factors influence the production, dispersal, survival, longevity, and germination of tree seeds. Researchers must have a general understanding of the effects of various environmental factors to select the most suitable location, time frame, experimental techniques, and types of sensors for field studies. The nature of these factors and the overall objectives of the study will also determine which environmental variables should be measured, and how frequently.

Environmental variables such as temperature and precipitation may be considered in the context of long-term average conditions, as ranges and extremes (climate), or as day-to-day conditions (weather). Furthermore, environmental variables can be viewed at three scales: macro (or regional) weather, site weather, and tree weather. The complexity involved in obtaining data increases as we go from macroclimate to tree weather.

*Macroclimate and synoptic weather conditions* can be obtained from the nearest Environment Canada or other government-operated weather stations, and may be adjusted for the elevation of the site of interest. Usually, climate data are summarized as monthly or annual values and include average, maximum, minimum, and extreme values for temperature, total precipitation, and derived data, such as degree-days and frost-free period. Wind speed and direction are available for a few locations. For some parts of British Columbia, annual temperature and precipitation summaries by subzone can be obtained from the biogeoclimatic ecosystem classification database (see Appendix C).

*Site climate and site weather conditions* involve on-site measurements of air and soil temperature, precipitation, humidity, wind speed and direction, solar radiation, and soil moisture. Monitoring usually requires an electronic datalogging system. Weather data may only be needed for a short time during an event of interest (e.g., pollen release); in this case, you may need hourly rather than daily summaries. However, to characterize the climate (averages and variation), 5–10 years of data collection are required. These data should be referenced to the nearest long-term weather stations to determine how different the period being measured may be from the “normal.”

*Tree weather* describes conditions in cones or flowers, or beside germinating seeds. Small, delicate sensors, such as thermocouples, are usually required to make these measurements. Variables of interest in regard to tree weather are temperature, radiation balance, and soil moisture (for germination). The data can be used to develop physically based models or regression models of tree conditions as a function of site conditions.
2.2 Designing an Environmental Monitoring Program

In designing an environmental monitoring program for a research site, you must first decide which variables you need to measure. For field germination studies, near-surface (0–5 cm depth) soil temperature and soil moisture are the important variables. However, soil temperature and moisture will be affected by a variety of other environmental variables. Soil temperature, for example, depends on soil moisture, solar radiation, wind speed, air temperature, soil texture, and surface colour. On the other hand, surface soil moisture depends on rainfall, solar radiation, evaporation, vegetation cover (transpiration), soil texture, and soil temperature. The humidity of the air and solar radiation can critically affect the initial establishment of germinants through its effects on soil evaporation and plant transpiration. Humidity also affects seed production through its effects on pollination. Slope and aspect affect temperature and moisture because they influence the solar radiation and rainfall reaching the surface. Wind is of interest primarily for studies of seed dispersal (see Section 4).

The frequency of environmental measurements will vary depending on the type of measurement. Light and temperature can vary rapidly and thus require frequent monitoring. Relatively stable site factors, such as soil type, soil pH, presence and type of duff layer, biogeoclimatic zone, elevation, slope, and aspect, may need only to be measured once.

Researchers sometimes rely on environmental data from the nearest weather station to provide data such as rainfall and daily minimum and maximum temperatures, but if the microclimate of the site is significantly different from that of the weather station it is advantageous to set up a small weather station at the site (Figure 2.1). Dataloggers can be used to continuously record a variety of environmental variables (Figure 2.2). Spittlehouse (1989) provides guidance on using dataloggers in the field and the accuracy that can be expected from such measurements. While it is tempting to collect large amounts of weather data on the assumption that somehow they will be useful, a few days of manual measurements under a range of weather conditions may be just as effective as installing electronic dataloggers on the site. The disadvantage of manual sampling, however, is that you may be able to demonstrate differences between treatments only when they are large.

Whatever the means of recording data, the complexity of environmental factors and their interactions necessitates careful planning of all field measurements. Four steps to developing an environmental monitoring program are illustrated here using an example of a study to determine conditions that initiate flowering.

1. Why do you need environmental data?
To determine weather conditions that initiate flowering, and their variation from year to year.

2. What data are needed?
Air and bud temperatures and solar radiation, from bud initiation through flowering (over 6 months or more). The year-to-year variation could be obtained by monitoring for many years, or by calculating regression equations that are based on weather data. Site weather conditions could be related to the nearest long-term weather station to provide the data that would be needed to drive the model. Ideally, a physically based model of bud/flower temperature as a function of site weather conditions and bud characteristics should be developed to allow portability to other sites with a minimum of calibration. Both methods require 2–3 years of on-site data for development and validation.
2.3 Methods for Measuring Environmental Factors

2.3.1 Soil temperature
Near-surface soil temperature (0–2 cm depth) can be easily measured, but measurements must be adequately replicated. Soil temperature varies substantially, not only horizontally and vertically, but temporally as well. Individual locations can be averaged by using a series of thermocouples connected in parallel. Data loggers are a convenient way to monitor the number of sensors required to assess the spatial and temporal variability. The diurnal trend of the near-surface temperature parallels the diurnal course of solar radiation; thus a reasonable approximation of the daily maximum temperature can be obtained by making the measurement about an hour after solar noon. An estimate of solar noon in your area is available on the Internet at http://www.crhnwscr.noaa.gov/grr/sunlat.htm. The average near-surface soil temperature (during the summer) can be approximated by measuring at about 8 a.m. solar time (4 hours before solar noon). A comparison of treatments with this approach requires that measurements be made under the same weather conditions. This manual sampling method will only be useful for showing differences larger than 5°C and should only be used to give an idea of trends.

Shade can significantly reduce solar radiation, resulting in a corresponding decrease in the near-surface temperature. On the other hand, shade also reduces night-time cooling. When solar radiation is reduced by over 60%, the surface temperature can be approximated by the air temperature at 1.5 m above the ground. For more information on forest soil temperature, see Stathers and Spittlehouse (1990).

2.3.2 Soil moisture
There is no easy way to obtain good soil moisture measurements, and the difficulty increases as one gets closer to the surface. Gravimetric sampling and time-domain reflectometry (TDR) measure soil water content (Hook et al. 1992), but further work is required to develop TDR probes and techniques. TDR requires substantial replication, and although it is usually done manually, it can be automated. Water content can be converted to soil water potential (or tension) using a soil water retention curve obtained...
from undisturbed soil samples analyzed by a commercial soil physics laboratory.

Gravimetric sampling is labour intensive and destructive. Some 5–10 replicates at each depth of interest are required. It is best to use a sharpened metal tube to take a soil core of known volume, rather than a grab sample. The sample is sealed in plastic bags, and returned to the laboratory for weighing and drying. Gravimetric samples are presented on either a weight of dry soil or a volumetric basis. The latter is the common approach and can be converted to soil water potential (or tension) using a soil water retention curve.

Gypsum or fibreglass soil moisture blocks can be used to measure soil moisture potential (or tension) in the 0 to -2.5 MPa (25 bars) range; they can be read manually or with a datalogger. Moisture blocks provide relatively coarse resolution, and require testing over several drying cycles before installation. They may have poor contact with substrates such as coarse sandy soil or partially decomposed organic material and cannot be used at soil depths shallower than 5 cm.

Tensiometers measure soil water potential in the 0 to -0.08 MPa (0.8 bars) range. They are usually read manually but can be automated. As with moisture blocks, they cannot be used at depths shallower than 5 cm. Soil water potential can also be obtained by equilibrating soil samples with the air or filter paper in a sealed container, then measuring the humidity of the air or filter paper. In situ measurements of soil water potential using soil psychrometers or hygrometers is extremely difficult, particularly in the top 15 cm of the soil. Further information on measuring soil moisture can be found in Schmugge et al. (1980).

2.3.3 Solar radiation (light)

Three ranges of the radiation spectrum are usually considered when assessing the light regime at the earth’s surface: ultraviolet radiation from 290 to 400 nm, photosynthetically active radiation (PAR) from 400 to 700 nm, and solar radiation from 300 to 3000 nm. Radiation above 3000 nm is called longwave or thermal radiation. Different sensors are required to measure each of these bands of energy. There is a good correlation between the energy in each band both above and below the canopy (Yang et al. 1993; Alados et al. 1996).

Radiant flux density is the energy in the light emitted, transmitted, or received per unit area (W/m²). Irradiance is the radiant flux density incident on a surface; emittance is the radiant flux density emitted by a surface.

The units and the instruments used for light measurement will depend upon the intent of the study. Some radiometers (for example the LI-COR model LI-189 radiometer) can be fitted with a variety of sensors to measure irradiance. A quantum sensor is used to measure photosynthetic photon flux density (PPFD) or PAR. A pyranometer (or radiometric sensor) is used to measure solar radiation. Photometric measurements using illumination units (lux or footcandles) should not be used in plant studies. Plants do not respond to the light spectrum in the same way as the human eye, so such measurements have no value unless the characteristics of the light source are precisely known (Salisbury and Ross 1992). PAR is usually the radiation measurement made when assessing physiological responses such as plant productivity (although other wavelengths may have specific photomorphogenic effects such as the induction of flowering or cold hardiness). PAR is commonly measured in units of μmol • m⁻² • s⁻¹. Special sensors are required to measure ultraviolet radiation, and various filters are available that modify sensor output to match the biological response of tissue. Longwave radiation sensors are not easy to use, so longwave estimates are usually obtained by subtraction of solar radiation from total radiation measurements (see Black et al. 1991).

The controlling influence of vegetation on the light regime will render a shaded surface more moist and cool than a bare surface. The amount of direct cover over the study area, the distance from the edge of openings, and the aspect of the edge will influence the light regime in openings. These influences can be estimated from measurements of above-canopy light and the amount of cover. When measuring irradiance under a plant canopy with uneven light levels, reasonable averages can be obtained by moving a small sensor repeatedly along a track (Figure 2.3), by using a long linear sensor, or by using many spot sensors. It is generally best to spend some time generating radiation interception curves with an intensive measurement program over a short period. These curves are used with continuously monitored above-canopy
radiation to give below-canopy data through the year. The variability or patchiness of the canopy may indicate that there is a range of light environments that must be quantified separately. Radiation rapidly decreases as canopy cover increases. The interception curve is of the form

\[ I = I_o e^{-KC} \]

where:
- \( I \) = the radiation at the forest floor,
- \( I_o \) = the radiation above the canopy,
- \( C \) = percent canopy cover (range 0–100), and
- \( K \) = an extinction coefficient (range 0.02–0.04).

Shaded and sunny areas of small openings and clear-cut edges can be determined using the formulae for length and direction of tree shadows at different times of the day and year (Sit 1992a).

Forest canopies change the quality as well as the intensity of light reaching the forest floor (Vezina and Boulter 1966; Atzet and Waring 1970; Ross et al. 1986; Messier et al. 1989). Figure 2.4 illustrates how the spectral distribution is changed due to plant foliage differentially absorbing and reflecting the various wavelengths. The relatively thick needles of coniferous trees transmit very little radiation and most radiation reaching the forest floor passes between needles and other gaps in the canopy. Consequently, the change in the shape of the spectrum is not as great as in hardwoods where more radiation passes through the thinner leaves. The biggest change is in the increase in the ratio of red (640–680 nm) to far-red (680–760 nm). It changes from 1.1–1.3 under clear skies above the canopy to 0.08–0.28 under hardwood forest canopies, and to 0.6–1.0 under coniferous canopies.

Light quality—the incident light spectrum—affects the germination of many conifer seeds (Section 7.1.2) and the production of female cones (Durzan et al. 1979). For measurement of irradiances under forest canopies, see Black et al. (1991) and Yang et al. (1993). The measurement of the total light spectrum

![Figure 2.3](image1.png) **Figure 2.3** Automatic tram system that moves back and forth over a 50 m span to determine the variation in short- and longwave radiation, and surface and air temperature under a forest canopy. The system is controlled by the datalogger in the tube hanging on the end. (System designed by R. Adams, B.C. Ministry of Forests.)

![Figure 2.4](image2.png) **Figure 2.4** Influence of forest canopy on the intensity and spectral distribution of solar radiation reaching the forest floor. The upper panel shows the incident radiation from a clear sky during the middle of the day. The lower panel (note the difference in scale) indicates that pine and maple canopies have greater absorption in the middle range (400–700 nm) than in the near infrared range (700–750 nm). (Based on data in Federer and Tanner 1966 and Vezina and Boulter 1966.)
at a site requires a portable spectroradiometer. Both Atzet and Waring (1970) and Floyd et al. (1978) conducted spectroradiometric analyses to determine the light-filtering capacity of coniferous forests. However, the changes in light quality can simply be measured with a portable red:far-red meter, since most of the canopy effects are due to the canopy cover shifting the ratio of red to far-red light.

### 2.3.4 Wind speed and wind direction

Wind, acting either directly or by wind-induced vibration, plays a major role in the distribution of pollen and seeds. Seeds of some species are very responsive to updrafts or vertical air movements (see Section 4.1.3). Wind speed is a vector quantity with attributes of direction and magnitude, although only the horizontal component is usually measured. Cup or propeller anemometers are generally used to monitor wind speed. They can be connected to a datalogger or have their own display. Many anemometers come with a wind direction indicator. Topography and vegetation cover affect wind speed and direction and care must be taken in locating the anemometer and wind vane. The sensors should be located 2–10 m above vegetation canopy and away from clearcut edges. Robust anemometers usually have stall speeds of 0.5 m s⁻¹ or greater. Low stall speed anemometers are required if you are interested in conditions at the edge of a clearing, in a small opening, or below the canopy. Hot-wire anemometers (commercial or home-made) can be used to measure wind flow around cones and flowers, but they are delicate and easily damaged. They can be monitored manually or with a datalogger. A discussion of wind dynamics and instrumentation can be found in Pearcy et al. (1989).

### 2.3.5 Precipitation

Rainfall can be reliably measured with tipping-bucket or storage gauge. The gauge should be located in an opening where a line projected from the top of the gauge to the top of the surrounding vegetation has an angle of no more than 45° to minimize any shading effects. Snowfall cannot be measured with a standard tipping-bucket gauge. Although gauges that melt the snow and can be monitored with a datalogger are available, they are expensive. A low-power, reliable sensor that measures the depth of snow on the ground can be used in conjunction with a datalogger.

### 2.3.6 Air temperature and humidity

A hygrothermograph in an instrument shelter (Stevenson Screen) located on the study site can record air temperature and relative humidity for up to a month before the chart requires changing. Electronic temperature and humidity sensors can be placed inside the Stevenson Screen. Smaller shields can be built or are available commercially, but some commercial varieties overheat under low wind speeds. Spot measurements can be made using aspirated or sling psychrometers.

### 2.3.7 Plant temperature

Obtaining temperature measurement in cones and buds requires extremely small sensors. Thermocouples (Figure 2.5) are the best option, being more robust and easier to make than thermistor or platinum resistance sensors. They are best monitored with a datalogger. Surface temperature can be measured using an infrared thermometer with a narrow field of view.

### 2.3.8 Canopy cover

Canopy cover is the environmental factor most immediately affected by forest harvesting activities and by silvicultural practices (see Section 8). It significantly affects the microclimate of a site, influencing the solar radiation, air and soil temperatures, wind speed, humidity and rainfall experienced at the ground (Hanley 1978). Canopy cover is also important because the position of vegetation within the canopy is used as a criterion for the relative dominance of individuals within a plant community (Section 3.2.3).

*Figure 2.5* A fine wire thermocouple is used to measure the temperature inside the leader of a young spruce tree. The thermocouple is monitored with a datalogger, as are the accompanying environmental sensors. The same technique can be used to measure cone temperature.
Canopy cover is often expressed as a percentage value, usually by species, growth form, or canopy stratum; in a dense or multilayered community, total vegetative cover may exceed 100%. The method chosen to measure canopy cover depends on the available technology and the type of site; some methods are suitable for low herbaceous vegetation or clearcut areas, while others are designed for forested areas. Bunnell and Vales (1990) present a comparison of different methods of measuring forest canopy cover.

Many researchers obtain percentage cover of different species and canopy layers with the visual-estimation technique (Mueller-Dombois and Ellenberg 1974). Cover can be estimated to the nearest percentage point or to the nearest 5th or 10th percentile. Cover estimates may be restricted to the plots being studied for germination or other responses, or may be used for more general descriptive purposes (such as describing the study site, Section 1.6). The visual-estimation method is especially suitable for grasslands or clearcuts, because of the low profile of the vegetation. Plot size for cover estimation averages about 1.0 m² (either circular or square), but may be smaller when working in exclusively herbaceous vegetation, or larger when working with tall shrubs and trees. A good guideline for plot size is that plot diameter should be approximately equal to the height of the vegetation being described.

Visual estimates are subject to personal bias, thus human error will introduce variability into the data (Bunnell and Vales 1990). This can be checked and corrected (calibrated) by other people working on the same project. It is also useful to have examples of how different spatial arrangements affect one’s perception of canopy cover. Figure 2.6, for example, compares different spatial arrangements of 50% canopy cover. These kinds of comparisons are especially useful when observers are not experienced in canopy estimation methods.

Canopy cover can be measured more objectively using a line-intercept method, which is suitable for woody plants, shrubs, and trees (Chambers and Brown 1983; Habitat Monitoring Committee 1996). A line or tape measure is stretched tightly across the vegetation between two stakes. The best sampling procedure is the stratified-random sample using a baseline and perpendicular transects (see Chambers and Brown 1983 for a sample layout). The length of the canopy intercept of each species along the line is measured from the tape or with a ruler. If the canopies overlap in layered vegetation, it may be desirable to measure each height layer separately. Transect lines should be 10–100 m in length. Many short lines are generally preferred to a few long lines; 5–10 transects are usually required for an adequate sample. Several cover values can be calculated:

\[
\text{percent cover for each transect by species} = \frac{\text{length intercepted by a species}}{\text{transect length}} \times 100;
\]

\[
\text{percent cover of a species by sampling unit} = \frac{\text{sum of all transect lengths intercepted}}{\text{total transect length sampled}} \times 100.
\]

While this method is generally precise and accurate (Cook and Stubbendieck 1986), it can also be time consuming.

Figure 2.6  Different spatial arrangements comprising 50% canopy cover. Some experience may be needed to estimate different proportions of cover.
Objective measurements of canopy cover can also be obtained with a point-intercept method (Owensby 1973; Levy and Madden 1993) or point-quadrat method (Chambers and Brown 1983). These methods use a point- or pin-frame, often consisting of 10 pins spaced 5 or 10 cm apart, with pins positioned vertically or at an inclined angle. The frame is positioned randomly within the sampling units or along a transect and a single pin lowered towards the ground. The first strike of any part of the vegetation canopy becomes a “hit.” Each “hit” is recorded by species or growth-form (Chambers and Brown 1983). The sample size required for statistical adequacy is usually 100–300 pins. Several cover values can be derived from this information: percentage canopy cover for each species or life-form (Chambers and Brown 1983); percent total canopy cover; and percentage vegetation cover by species. The user should be aware that the line is the sample unit, so it is better to have fewer points per line and more lines, rather than vice versa (Bonham 1989). The frame should be held vertically; if the frame is at an angle, the number of intercepts may increase and overestimate the cover.

In woodland areas, other instruments such as the moosehorn and the spherical densiometer are frequently used to measure tree canopy cover (Lemmon 1957; Bunnell and Vales 1990). The moosehorn is a point-intercept method where the canopy is viewed through a screen and coincidences between vegetation and dots on the screen are tallied. The spherical densiometer has a curved mirror with a grid that reflects the overstorey at a particular point, then provides an estimate of the relative amount of the grid covered by vegetation. At each location, four readings (facing north, east, south, and west) are recorded and averaged.

A canopy analyzer uses measurements from a fisheye optical sensor placed above and below the plant canopy; in this way canopy transmittance data can be used to calculate the leaf area index and the mean leaf inclination angle (Chen et al. 1991; Welles and Norman 1991). The canopy analyzer functions in a variety of sky conditions, with overcast being the best; the instrument can be used in canopies ranging in size from short grasses to forests.

The area of leaf per unit area of ground (leaf area index - LAI) is another measure used to quantify canopy cover. It is measured by sampling the foliage or by using light penetration techniques (Gholz et al. 1976; Smith et al. 1993; Fassnacht et al. 1994; Chen 1996). In the former method, all the foliage in the shrub and herb layers is removed from 5–10 samples of known area (usually 1 m²). The area of the leaves is then measured using an image analyzer. All the leaves from a tree (or from a representative branch in each whorl) are sampled and leaf area measured with an image analyzer. Trees of different diameter at breast height (dbh) are sampled to produce a dbh/leaf area relationship (or sapwood cross-sectional area/leaf area) which is then used with stand dbh distribution to calculate tree LAI. Leaf area can be determined using light sensors such as the ceptometer and the LAI-2000. Both measure the “effective” leaf area and must be corrected for leaf clumping to get the true leaf area index (Smith et al. 1993; Fassnacht et al. 1994; Chen 1996). These sampling techniques can be used to determine how leaf area changes with height and to calculate foliage area density.

The canopy can be photographed from ground level using a camera fitted with a hemispherical or fisheye lens with a 180° field of view. Film exposure must be standardized (Chen et al. 1991). The resulting photographic negatives, prints, or slides can be digitized, and then analyzed by a computer program to accurately measure canopy cover above the point of measurement. Available computer programs include SOLARCALC (Chazdon and Field 1987), Gli (Canham 1988), SUNSHINE (Smith and Somers 1991), and HEMIPHOT (ter Steege 1993). This photographic method is suitable in herbaceous, scrub, forested, or mixed cover, but has the drawback that considerable office time is required to obtain cover estimates. These same programs also model solar radiation input for the point at which the photograph was taken (see Section 2.3.3), but the cover estimates require fewer assumptions.

2.3.9 Soil variables
Soil nutrient levels are important because they affect seed production, germination, and seedling growth. Three principal methods are used to diagnose nutrient deficiencies: deficiency symptoms, soil chemical analysis, and foliar analysis. (For other methods see Morrison 1974.) Soil chemical analysis has some value for diagnosing site nutrient status on sites where
foliage sampling is impractical. There are major problems with conducting soil chemical analysis in forest soils. Typically, the root zone is not homogeneous, often containing dissimilar horizons that may yield different analytical values. Nutrient standards for forestry soils are not available, and criteria cannot be extrapolated from one kind of soil to another. It can be problematic to integrate these disparate results to determine the nutrient status of the composite soil profile.

The high variability of some soils may require a large number of samples. The most useful routine soil chemical analyses for forest soil fertility in B.C. are likely to be: pH, organic carbon concentration, and total nitrogen concentration (Watts [editor] 1983).

Oxygen is usually a limiting factor only in waterlogged soils, where water may fill pore spaces. Oxygen is difficult to measure in the field, but under suitable conditions, an oxygen electrode may be used. This technique uses glass electrodes that are delicate and easily broken, and is not generally suitable for field studies. It is primarily designed for laboratory studies, but can be set up and operated adjacent to a study site. A key requirement is constant-temperature water, obtained from a thermoregulated circulator or a large-reservoir flow-through system. Some instruments can be configured to determine oxygen indirectly by heating the sample in a stream of inert gas and converting all oxygen-containing gases to carbon monoxide or carbon dioxide. Refer to Pearcy et al. (1989) for further information on such methods.
SECTION 3  NATURAL SEED PRODUCTION

O sweet spontaneous
earth how often have
the
doting

    fingers of
prurient philosophers pinched
and
poked
thee,
has the naughty thumb
of science prodded
thy

    beauty .
(e.e. cummings)

3.1 Background

The path to the production of a viable seed begins
with the growth and development of reproductive
buds, continues with pollination and fertilization,
and ends with embryo development and seed matu-
ration. Throughout all these developmental stages
losses occur for a variety of reasons; losses may be
due to environmental factors, or may result from
various diseases and animal predators that attack
cones and seeds. Researchers investigating tree seed
production must be able to assess which and to what
extent these factors limit natural seed supplies.

Angiosperms characteristically produce seeds an-
nually, but production can vary considerably from
year to year (Table 3.1). Most conifers do not produce
collectable crops every year (Table 3.2), a phenomenon
called periodicity. Mature cones are produced at in-
tervals ranging from 3 to 10 years, and sometimes as
infrequently as every 15 years. Crop yields vary in
different years, and in poor crop years, the quality
of seeds also tends to be poor (Edwards 1980; Caron
and Powell 1989a, 1989b). Depending upon the
species, conifer seed production varies in length and
complexity of the production cycle (Figure 3.1), repro-
ductive success (due to different sexual mechanisms),
and the timing of natural seedfall (Zobel 1979).

The reproductive structures of trees are derived
from reproductive buds. The time of initiation of
male and female reproductive buds can vary from
year to year due to factors such as the relative abun-
dance of seed trees (trees/ha) (Smith et al. 1988) and
tree age (Caron and Powell 1989b). Natural seed pro-
duction is rare in trees younger than 10 years.
Generally, the volume of seeds produced increases as
the tree ages. Bergsten (1985), however, found no bio-
logical differences between mature Scots pine seeds
obtained from young stands and those collected from
old stands. Environmental conditions, such as tem-
perature, drought (Eis 1973a, 1976), and nutrient
availability (Heidmann 1984), affect reproductive bud
production. Environmental stresses can reduce the
**Table 3.1** Seed production characteristics of hardwoods native to British Columbia. Sources: Schopmeyer (1974); Pojar and MacKinnon (1994); Wyckoff and Zasada [1998]; Zasada et al. [1998]

<table>
<thead>
<tr>
<th>Species Common name</th>
<th>Tree type</th>
<th>Flowers (description)</th>
<th>Flowers mature (month)</th>
<th>Seeds mature (month)</th>
<th>Fruit (description)</th>
<th>Average # seeds/fruit</th>
<th>Interval between crops</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acer macrophyllum</em></td>
<td>Monoecious; imperfect flowers</td>
<td>Greenish yellow (3 mm across); numerous in hanging cylindrical cluster; male and female in different parts of crown</td>
<td>Apr–May</td>
<td>Ripen Sept–Oct; disperse Oct–Jan</td>
<td>Paired winged seeds (samaras); 3–6 cm long</td>
<td>2 (1 / samara); no endosperm</td>
<td>1 year</td>
</tr>
<tr>
<td><em>Alnus rubra</em></td>
<td>Monoecious</td>
<td>Drooping reddish male catkins (5–12 cm); female catkins are woody cones (2 cm)</td>
<td>Male, previous fall; female, Feb–May</td>
<td>Ripen Aug–Sept; disperse Aug–spring</td>
<td>Brown cones (2 cm) remain over winter; contain oval, winged nutlets</td>
<td>50–100 seeds / cone no endosperm</td>
<td>4 years</td>
</tr>
<tr>
<td><em>Arbutus menziesii</em></td>
<td>Monoecious; perfect flowers</td>
<td>White, urn-shaped (7 mm), in large drooping clusters</td>
<td>Apr–May</td>
<td>Fall</td>
<td>Orange-red berry-like (1 cm), surface finely granular</td>
<td>20 hard, angled seeds + endosperm</td>
<td>1 year</td>
</tr>
<tr>
<td><em>Betula papyrifera</em></td>
<td>Monoecious</td>
<td>Male stamine flower and female strobile (2–4 cm); break up at maturity</td>
<td>Male, previous fall; female, Apr–June</td>
<td>Ripen Aug–Sept; disperse Aug–spring</td>
<td>Nutlets with wings broader than body</td>
<td>Numerous no endosperm</td>
<td>2 years</td>
</tr>
<tr>
<td><em>Cornus nuttallii</em></td>
<td>Monoecious; perfect flowers</td>
<td>Small (3 mm) white in tight clusters surrounded by 4–6 white showy bracts (2–7 cm)</td>
<td>Always in spring; often in fall</td>
<td>Ripen late summer/fall</td>
<td>Clusters of bright red “berries” (1 cm); globular or ovoid drupes</td>
<td>1–2 / stone; + endosperm</td>
<td>2 years</td>
</tr>
<tr>
<td><em>Fraxinus latifolia</em></td>
<td>Dioecious</td>
<td>Small (3 mm) male (yellowish) and female (greenish) flowers in bunched clusters on twigs</td>
<td>Appear before leaves</td>
<td>Late summer/fall</td>
<td>Paddle-shaped, samara (3–5 cm) in large clusters on female trees</td>
<td>1 / samara + endosperm</td>
<td>1 year</td>
</tr>
<tr>
<td><em>Malus fusca</em></td>
<td>Monoecious; perfect flowers</td>
<td>White to pink fragrant blossoms (2 cm); 5–12 in flat-topped clusters</td>
<td>Apr–June</td>
<td>Late fall</td>
<td>Yellow to reddish small (10–15 mm) fleshy pomes</td>
<td>3–5 carpels 1–2 seeds / carpel + endosperm</td>
<td>2–4 years</td>
</tr>
<tr>
<td><em>Populus balsamifera</em> ssp. <em>balsamifera</em></td>
<td>Dioecious</td>
<td>Male and female catkins</td>
<td>Apr</td>
<td>June–July</td>
<td>2-valved capsules; not hairy</td>
<td>Numerous; no endosperm</td>
<td>1 year</td>
</tr>
<tr>
<td><em>Populus balsamifera</em> ssp. <em>trichocarpa</em></td>
<td>Dioecious</td>
<td>Male catkins (2–3 cm) with 40–60 stamens / flower, female catkins (8–20 cm) with 3 stigmas / flowers</td>
<td>Appear before leaves (Apr–June)</td>
<td>May–July</td>
<td>Round, green hairy capsules that split into 3 parts; seeds covered with white fluffy hairs</td>
<td>Numerous; no endosperm</td>
<td>1 year</td>
</tr>
<tr>
<td>Species</td>
<td>Common name</td>
<td>Tree type</td>
<td>Flowers (description)</td>
<td>Flowers (month)</td>
<td>Seeds mature (month)</td>
<td>Fruit (description)</td>
<td>Average # seeds/fruit</td>
</tr>
<tr>
<td>-------------------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>-----------------------------------------------------------</td>
<td>-----------------</td>
<td>---------------------</td>
<td>--------------------------------------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>Populus tremuloides</td>
<td>quaking aspen</td>
<td>Dioecious</td>
<td>Male catkins (2–3 cm); female catkins (4–10 cm)</td>
<td>Apr–May</td>
<td>May–June</td>
<td>Catkins of tiny, greenish capsules covered with cottony down</td>
<td>2–7 / capsule; 77–500 seeds / catkin no endosperm</td>
</tr>
<tr>
<td>Prunus emarginata</td>
<td>bitter cherry</td>
<td>Monoecious; perfect flowers</td>
<td>5–10 white to pinkish flowers (10–15 cm) in flattopped cluster</td>
<td>Apr–June</td>
<td>July–Sept</td>
<td>Dark red drupes (1 cm)</td>
<td>1 / drupe</td>
</tr>
<tr>
<td>Quercus garryana</td>
<td>Garry oak</td>
<td>Monoecious</td>
<td>Tiny inconspicuous flowers; male, in hanging catkins; female, single or in small clusters</td>
<td>Feb–May</td>
<td>Aug–Dec</td>
<td>Acorns (2–3 cm) in shallow, scaly cups</td>
<td>1 / acorn; cotyledons only, no endosperm</td>
</tr>
<tr>
<td>Rhamnus purshiana</td>
<td>cascara</td>
<td>Monoecious</td>
<td>Loose clusters of 5–12 tiny, yellowish-green flowers</td>
<td>Apr–July</td>
<td>July–Sept</td>
<td>Purplish-black, round berrylike drupe</td>
<td>2–3 nutlike seeds / drupe + endosperm</td>
</tr>
<tr>
<td>Salix amygdaloides</td>
<td>peach-leaf willow</td>
<td>Dioecious</td>
<td>May–June</td>
<td>July</td>
<td>Small, 2-valved capsule contains hairy seeds</td>
<td>14–18 seeds / capsule</td>
<td></td>
</tr>
<tr>
<td>Salix bebbiana</td>
<td>Bebb's willow</td>
<td>Dioecious</td>
<td>Apr–June</td>
<td>May–June</td>
<td>2-valved capsule; 24–48 capsules/catkin</td>
<td>5–7 seeds / capsule; 144–311 seeds per catkin</td>
<td></td>
</tr>
<tr>
<td>Salix discolor</td>
<td>pussy willow</td>
<td>Dioecious</td>
<td>Staminate catkins soft, silky</td>
<td>May</td>
<td>2-valved capsule</td>
<td>8–12 / capsule</td>
<td></td>
</tr>
<tr>
<td>Salix exigua</td>
<td>sandbar willow</td>
<td>Dioecious</td>
<td>May–July</td>
<td>May–June</td>
<td>2-valved capsule</td>
<td>15–36 / capsule</td>
<td></td>
</tr>
<tr>
<td>Salix lucida ssp.</td>
<td>kasiandra</td>
<td>Dioecious</td>
<td>Apr–May</td>
<td>June–Aug</td>
<td>2-valved capsule</td>
<td>12–20 / capsule</td>
<td></td>
</tr>
<tr>
<td>Salix scouleriana</td>
<td>Scouler's willow</td>
<td>Dioecious</td>
<td>Apr–June</td>
<td>May–July</td>
<td>2-valved capsule</td>
<td>2-valved capsule</td>
<td></td>
</tr>
</tbody>
</table>
### Table 3.2: Seed production characteristics of conifers native to British Columbia (Eremko et al. 1989). (Cones refer to female cones only.)

<table>
<thead>
<tr>
<th>Species Common name</th>
<th>Cone length (cm)</th>
<th>Cone-bearing age (years)</th>
<th>Years between crops</th>
<th>Viable seeds per hectolitre of cones</th>
<th>Position of cones in crown</th>
<th>Ease of cone detachment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abies amabilis amabilis fir</td>
<td>9–13</td>
<td>20</td>
<td>2–3</td>
<td>30 389</td>
<td>Top ¼</td>
<td>Difficult</td>
</tr>
<tr>
<td>Abies grandis grand fir</td>
<td>5–12</td>
<td>50</td>
<td>2–3</td>
<td>50 776</td>
<td>Top ¼</td>
<td>Difficult</td>
</tr>
<tr>
<td>Abies lasiocarpa subalpine fir</td>
<td>6–12</td>
<td>20</td>
<td>2–4</td>
<td>40 582</td>
<td>Top ¼</td>
<td>Difficult</td>
</tr>
<tr>
<td>Chamaecyparis nootkatensis yellow-cedar</td>
<td>0.5–1.5</td>
<td>Unknown</td>
<td>4 or more</td>
<td>93 965</td>
<td>Throughout</td>
<td>Easy</td>
</tr>
<tr>
<td>Larix laricina tamarack</td>
<td>1.5</td>
<td>40</td>
<td>3–6</td>
<td>32 000</td>
<td>Non-shaded part of crown</td>
<td>Moderate</td>
</tr>
<tr>
<td>Larix occidentalis western larch</td>
<td>2–3</td>
<td>25</td>
<td>1–10</td>
<td>119 312</td>
<td>Non-shaded part of crown</td>
<td>Moderate</td>
</tr>
<tr>
<td>Picea glauca white spruce</td>
<td>3–6</td>
<td>40</td>
<td>6</td>
<td>347 163</td>
<td>Top ½</td>
<td>Moderate</td>
</tr>
<tr>
<td>Picea mariana black spruce</td>
<td>2.5</td>
<td>10</td>
<td>4 or more</td>
<td>108 000</td>
<td>Top ¼</td>
<td>Difficult</td>
</tr>
<tr>
<td>Picea sitchensis Sitka spruce</td>
<td>5–10</td>
<td>25–40</td>
<td>3–4</td>
<td>194 270</td>
<td>Top ½</td>
<td>Moderate</td>
</tr>
<tr>
<td>Pinus albicaulis whitebark pine</td>
<td>3–8</td>
<td>20–30</td>
<td>3–5</td>
<td>515</td>
<td>Throughout</td>
<td>Difficult</td>
</tr>
<tr>
<td>Pinus contorta shore pine</td>
<td>3–6</td>
<td>15–20</td>
<td>2–4</td>
<td>coast: 176 660</td>
<td>Throughout</td>
<td>Difficult unless frozen</td>
</tr>
<tr>
<td>Pinus flexilis limber pine</td>
<td>7–20</td>
<td>20–30</td>
<td>2–4</td>
<td>6 454</td>
<td>Throughout</td>
<td>Moderate</td>
</tr>
<tr>
<td>Pinus monticola western white pine</td>
<td>10–25</td>
<td>20</td>
<td>3–7</td>
<td>7 687</td>
<td>Top ¼</td>
<td>Moderate</td>
</tr>
<tr>
<td>Pinus ponderosa ponderosa pine</td>
<td>7–9</td>
<td>12–16</td>
<td>4–6</td>
<td>31 522</td>
<td>Throughout</td>
<td>Difficult</td>
</tr>
<tr>
<td>Thuja plicata western redcedar</td>
<td>1–2</td>
<td>20–30</td>
<td>2–4</td>
<td>897 837</td>
<td>Throughout</td>
<td>Easy</td>
</tr>
<tr>
<td>Tsuga heterophylla western hemlock</td>
<td>2–3</td>
<td>25–30</td>
<td>3–4</td>
<td>366 698</td>
<td>Throughout</td>
<td>Easy</td>
</tr>
<tr>
<td>Tsuga mertensiana mountain hemlock</td>
<td>2–8</td>
<td>30</td>
<td>3–6</td>
<td>356 428</td>
<td>Top ½</td>
<td>Easy</td>
</tr>
</tbody>
</table>
number of reproductive buds or, in other cases, can stimulate prodigious production of cones. Plant growth regulators (PGR) such as gibberellins have been used to increase cone production in conifer seed orchards (Ross and Bower 1989; Ross 1991), but PGR levels are difficult to alter, for logistical reasons, in natural stands.

Pollen, produced in male cones or anthers, is transported to female cones or flowers in the process of pollination. Successful pollination results in the fertilization of ovules; ovules then develop into seeds. Reduced pollination efficiency may be due to low pollen-cloud densities (few pollen-cone buds initiated), climatic conditions (e.g., rain, frost), or the presence or absence of pollen vectors. In conifers, which are wind pollinated, the absence of wind, or barriers to wind may inhibit pollination. Thus, the positioning of cones relative to tree height or relative to the windward and leeward sides of a tree can influence the frequency of filled seeds (Smith et al. 1988). In angiosperms, animals (insects, birds, and mammals) usually are the primary vectors of pollination. Pollen is sometimes dispersed by wind, but generally angiosperm pollination is less affected by climatic variables, although extreme conditions (cold temperatures, heavy rain) may still affect pollination success.

Fertilization efficiency may be reduced due to poor female cone production, self-pollination (which often results in embryo abortion), lack of pollen tube growth, or freezing temperatures (Shearer and Carlson 1993). Some conifers (e.g., Douglas-fir) can produce seeds (megagametophyte, but no embryo) without fertilization, but other conifers (e.g., pines) require the presence of pollen to form seeds (Owens and Molder 1984b). Additional background on the sexual reproduction of conifers may be found in Owens and Molder (1984a, 1984b, 1984c, 1984d, 1985).

Once fertilized, seeds may fail to mature due to abortion (which may be caused by self-incompatibility, insects, or disease), or because of climatic conditions during embryo development, particularly cool, cloudy weather during the summer (Eis 1976; Zasada et al. 1978). Some conifers do not shed their seeds when they mature in the fall, and instead may retain

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**Figure 3.1** Typical development and maturation cycles of British Columbia conifer seeds (Leadem 1996, adapted from Eremko et al. 1989). Most angiosperms exhibit a reproductive cycle similar to that shown for Douglas-fir, redcedar, true firs, and hemlocks.
their seeds in the cones several years, a phenomenon referred to as serotiny (see Section 3.5.1).

Further information on tree seed biology may be found in Leadem (1996).

Seed production studies are usually undertaken to determine:
• the quantity and quality of seeds that may be produced relative to some variable, or
• the reasons for seed loss.

Other, more specific objectives may include:
• to predict the frequency of good seed crops (e.g., relative to climatic variables);
• to relate seed production to stand, tree, or crown characteristics;
• to examine the relationship between pollen abundance and filled seeds per cone;
• to relate the number of seeds per cone to cone age or cone size;
• to determine the relationship between the number of seeds in the cone half-face and the total number of filled seeds per cone;
• to determine the date of cone and seed maturation; or
• to establish the relation between seed quality and collection date, or cone handling methods.

Several examples of seed production studies are described as case studies in Section 3.8.

3.1.1 Collecting stand and study plot information

Before conducting seed production studies in natural stands, data should be collected on the tree and stand characteristics known to influence natural seed production. Examples are:
• density (number of seed trees per hectare);
• spatial arrangement of seed trees;
• age of seed trees;
• evidence of past production;
• evidence of animal use (e.g., squirrel caches, cones that have been broken or split);
• height and diameter at breast height (dbh);
• assessments of the general health and vigour of the crowns; and
• basal area values (in square metres per hectare).

Specific selection criteria may be included, for example the basal area of all Engelmann spruce trees with dbh of 25.4 cm and larger, because trees of this size probably would be sufficiently mature to produce seeds (McCaughey and Schmidt 1987).

Once a site has been selected, data on individual trees may be collected. Examples of the data that could be included are (Alexander et al. 1986):
• dbh to the nearest 0.25 cm (trees 9.1 cm dbh and larger);
• total height, to the nearest 0.15 m;
• crown class;
• species;
• average length of live crown to the nearest 0.15 m (average of four sides); and
• average width of live crown to nearest 3.0 cm (average of two measurements).

3.1.2 Determining sample size

The choice of sample size, such as the number of seeds to sample per tree, can be made by applying statistical efficiency calculations to a preliminary set of measurements (Sokal and Rohlf 1981; Ager and Stettler 1983). See also Stauffer (1981, 1982) for sample size tables prepared specifically for forestry applications.

Sample sizes for measuring cone characteristics will depend on the species and the sites from which the cones were collected. Carlson and Theroux (1993) randomly selected 20 cones each from some subalpine larch, hybrid larch, and western larch collections. Only five cones were selected from six other western larch collections because initial sampling error estimates indicated that five cones would be adequate. Sample sizes for seed measurements should also be determined before the study. For a study of western larch and subalpine larch, length, width, and thickness were measured on only 10 seeds randomly selected from each lot; initial sampling estimates indicated this would enable standard errors to within 20% of the mean (95% confidence) (Carlson and Theroux 1993).

Environmental changes may result in year-to-year variations in cone and seed measurements. Ponderosa pine seeds collected in 1971, 1974, and 1976 showed negligible differences in seed weight, length, and width when comparisons were made within the same year. However, differences were found in all three measures when year-to-year variations were removed by adjusting values to be relative to those observed in 1971 (Ager and Stettler 1983).
3.2 Predicting Natural Seed Yields

It is often desirable to be able to predict the occurrence of natural seed production, to better understand what factors promote seed crops, to determine whether seed production will be great enough to merit collection of the crop, or to provide advance notification for organizing pre-collection activities. In the sense used in this section, a distinction is made between prediction and correlation. Prediction is the objective of these studies (we are trying to predict natural seed production) and correlation is the means to do so (correlations with various variables are used to predict the size of the crop).

3.2.1 Correlation with weather variables

Many models for predicting the size of natural seed crops have been developed, and those based on climatic variables indicate that the influence of weather conditions may be cumulative. In Douglas-fir and grand fir, a cool, cloudy summer 24–26 months before crop maturation appears to be a prerequisite for abundant lateral bud initiation. These conditions must be followed by cold, sunny weather through the winter (20–28 months before maturation); a wet April (16 months in advance) to promote lateral bud differentiation; and a warm, dry, sunny June before pollination (14 months before maturation) (Eis 1973a, 1973b). (See Figure 3.2 for a summary.) The importance of dry summers to floral initiation has also been demonstrated in other species, such as spruce, larch, and ponderosa pine (Eis and Craigdallie 1983).

For estimates of Douglas-fir, grand fir, and western white pine cone crops, Eis (1973a, 1976) counted cones on one side of mature trees in July. Counting was done using 10-power binoculars mounted on a tripod at a permanent station that offered a good view of the crown. Cone counts were multiplied by conversion factors (obtained by comparing binocular observations with physical cone counts on felled trees). Weather variables were derived from various expressions of temperature, precipitation, sunshine, and wind velocity. Starting 29 months (41 months for western white pine) before the cones matured, cone estimates were correlated with all monthly meteorological parameters. Where several meteorological variables were important in the same month, the data were combined and analyzed by stepwise, forward, multiple regressions.

Caron and Powell (1989b) correlated annual production of black spruce seed cones with warm weather in early May and early July and with low June rainfall, all in the year preceding maturation. Cone production data were recorded branch-by-branch during later spring. Seed-cone estimates of previous crops were obtained from a combination of (1) cones persisting on the trees, (2) stubs and basal cone scales left on the bearing shoots where squirrels had removed cones, and (3) cones or stubs on nearby shoots of comparable size and position within the crown when shoots of bearing type had been removed.

Mosseler (1992) used accumulated growing degree-days (GDD) to predict when cones of black spruce and white spruce could be collected without

![Figure 3.2: Climatic conditions required for cone crop production in Douglas-fir (Eremko et al. 1989).](image-url)
adversely affecting seed quality. Accumulated GDD is a cumulative sum of the degrees of temperature above 5°C counted on each day that the daily mean temperature exceeds the 5°C threshold. In this study, Mosseler based the GDD on the simple mean of the maximum and minimum daily temperatures recorded at the Atmospheric Environment Service (Environment Canada) weather station nearest to each site. Cones were harvested at intervals of 100 GDD beginning at about 600 GDD. Mosseler found that natural seed release in white spruce occurred between 1200 and 1250 GDD. Cones from black spruce can be collected as early as 900 GDD and white spruce as early as 1100 GDD without significant losses in seed yield or quality. Similar results were found for white spruce in Alaska (J. Zasada, pers. comm., 1996).

Note that when attempting to correlate environmental factors to seed production, it is important to place sensors as near as possible to where pollen and seed cones are produced to ensure you are monitoring the conditions actually present in the canopy. Also, because comparable events in the reproductive cycle are not always synchronous, male and female flowers, for example, may not experience the same climatic conditions, so the environmental effects may be different. In paper birch, male flowers are induced in May before bud burst and thus must depend on resources stored in overwintering materials. Female flowers develop in late June to early July, so they are able to draw on current metabolites for their growth (Macdonald and Mothersill 1987).

### 3.2.2 Correlation with aspect and slope

Aspect and slope can significantly affect cone production, especially in northern regions. For example, black spruce trees growing on southerly aspects bore 2.5 and 5 times more seed cones and pollen cones, respectively, than trees growing on northerly aspects (Simpson and Powell 1981). Variations in slope and aspect can be difficult to depict, yet Simpson and Powell effectively conveyed their results by using concentric circles to show the percentage of cones produced in all compass directions (see Figure 3.3).

### 3.2.3 Correlation with crown size and crown class

In a closed canopy, the crowns of the trees forming the canopy are touching and intermingled so that light cannot directly reach the forest floor. However, dominant trees have crowns extending above the general level of the canopy and thus receive full light from above and partly from the side. The crowns of codominant trees, which form the general level of the canopy, receive full light from above, but comparatively little light from the sides. The relatively more favourable light environment for tree crowns in the upper canopy appears to enhance the cone production of dominant and codominant trees.

For example, dominant and codominant crown classes of Engelmann spruce produced three-quarters or more of the total seedfall in an experimental forest in the Colorado Rocky Mountains (Alexander et al. 1986) (see Case Study 1, Section 3.8). Also in black spruce, dominant trees produced almost three times

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**Figure 3.3** Percentages of black spruce trees (concentric circles) 8–12 years old from seed, growing on slight (2–12%) slopes and on various aspects (Simpson and Powell 1981), which in 1980 bore: (a) more than five, (b) more than 15, and (c) more than 25 pollen cones.
as many cones as codominant or the intermediate trees. Intermediate trees, on the other hand, produced about twice as many seeds per cone as dominant trees (Payandeh and Haavisto 1982) (see Case Study 4, Section 3.8). Note, however, in exceptionally good cone years, trees in all crown classes produce cones, not just the dominant and codominant trees (J. Zasada, pers. comm., 1996).

One possible reason for the periodicity observed in conifers is the substantial drain that cone production evidently places on the tree’s resources. In Douglas-fir, decreased needle, shoot, and xylem ring growth was noted in good seed years in the trees that regularly produced cones (Tappeiner 1969). No such reductions were seen in trees that did not produce cones. Similar effects have been seen in grand fir, western white pine (Eis et al. 1965), subalpine fir, and mountain hemlock (Woodward et al. 1994).

Seki (1994) wanted to know the specific location of resources used to produce seeds in *Abies mariesii*, so he studied the allometric relationship between cone production and the productivity of the entire crown and of cone-producing branches. He concluded that total cone production was related to the square of the trunk diameter just below the lowest living branch ($D_B^2$ in Figure 3.4). He concluded that cone production was not related to the total amount of dry matter in the tree, but rather to the amount of dry matter accumulated in the neighbouring trunk and branches adjacent to cone production sites. Thus, cone production per branch depends on the resource status of a branch, to which at least part of the resources may be imported from other branches or the trunk for local cone production. In this way, the investment in seed production in individual branches may not necessarily cost the whole plant its vegetative growth or future survival.

### 3.2.4 Sampling methods using bud counts

Eis (1973b, 1976) developed a sequential sampling method to estimate white spruce and western white pine cone crop potential in the fall preceding the seed year. The method is based on the cumulative total count of female buds from one branch per tree collected from

**Figure 3.4** Position of measurement for trunk diameters, the diameter of the base of a branch, and main axes for estimating of the number of cones (Seki 1994). Allometric relationships between various parts of a tree can be used for relating cone production to tree dimensions.
the third whorl from the top. Bud counts from three terminal nodes on a branch of the fourth or fifth stem node may also be used, but with slightly lower accuracy. Trees should be 45–80 years old, 15–18 m high, of dominant class, with well-developed crowns. The observer must be able to distinguish reproductive buds (both male and female) from vegetative buds (identification based on general morphology, location in the crown and along the branch, and colour). When the cumulative bud count falls between given limits, cone crop potential can be classified with 80% probability, and no further samples are required.

Male pollen buds can be used in birch and alder to indicate the next year’s seed production. Male buds are easily identifiable any time after September, and can be counted from the ground to provide reasonable estimates of female catkin production the following spring (J. Zasada, pers. comm., 1996).

A multistage variable probability sampling method, originally developed to estimate seed orchard efficiencies, could be applied to assess seed production in natural stands. Bartram and Miller (1988) first implemented a standard multistage approach in many seed orchards over several years. The effectiveness of this approach was evaluated against several alternative methods using the efficiency data initially collected for the study. Refer to the original paper for an example using this methodology in coastal Douglas-fir seed orchards in British Columbia. Mattson (1978) also suggested a multistage approach to evaluate red pine cone and seed production. In this scheme, the first stage is based on weather factors and the second stage on insect predators.

### 3.2.5 Scales for rating cone crops

Crop rating is an operational assessment procedure used by the B.C. Ministry of Forests to determine whether developing cone crops are collectable (Eremko et al. 1989). Suitable stands are located, and the relative size of developing cone crops is assessed in late June or early July. A visual assessment is made of the relative number of cones in the cone-bearing portion of dominant and co-dominant tree crowns. The number of cones on each cone-bearing tree and percentage of trees bearing cones in the stand are also assessed. Observations are grouped into classes depending on the relative number of cones observed on the tree (Table 3.3).

<table>
<thead>
<tr>
<th>Class</th>
<th>Rating</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>none</td>
<td>No cones</td>
</tr>
<tr>
<td>2</td>
<td>very light</td>
<td>Few cones on less than 25% of the trees</td>
</tr>
<tr>
<td>3</td>
<td>light</td>
<td>Few cones on more than 25% of the trees</td>
</tr>
<tr>
<td>4</td>
<td>light</td>
<td>Many cones on less than 25% of the trees</td>
</tr>
<tr>
<td>5</td>
<td>medium a</td>
<td>Many cones on 25–50% of the trees</td>
</tr>
<tr>
<td>6</td>
<td>heavy a</td>
<td>Many cones on more than 50% of the trees</td>
</tr>
<tr>
<td>7</td>
<td>very heavy a</td>
<td>Many cones on almost all of the trees</td>
</tr>
</tbody>
</table>

* Crops rated as class 5 or higher are generally considered collectable.

The rating of potential cone crops is highly subjective and dependent on the surveyor’s experience. The number of cones produced—and their distribution through the crown—varies considerably with tree species. Thousands of cones can constitute “many” on a spruce tree; the same number could be classed as “few” on a mature cedar.

A method based on seedfall data has been used in Oregon and California for rating cone crops of *Abies*, *Pseudotsuga*, *Tsuga*, and *Chamaecyparis* (Zobel 1979). Seeds were collected from traps approximately once a month over a 2-year period. The monthly trap samples from a site were usually combined, except where seed production was high enough to separately count individual traps. Basal area of each tree species over 15 cm dbh in each stand was measured using a wedge prism, with each trap as a sample point. Seed production effectiveness of a site was expressed as the annual seedfall per square metre of basal area of each species.

In another study, seed production of Engelmann spruce was based on seeds captured in traps and grouped into categories (Table 3.4).

Note that there may be some discrepancy between the cone crop rating and the number of seeds collected in seed traps. Such discrepancies can occur...
Section 3 Natural Seed Production

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in areas where there is heavy predation of cones by squirrels. Thus, generally it is best to count when cones have attained maximum physical dimensions, and before squirrels begin to harvest.

Cone crop estimates also can be obtained by direct sampling of cone-bearing regions or fertilized flowers. For example, cone crops of eastern redcedar (Juniperus virginiana) were estimated by multiplying the average number of cones per sample branch (5–10) in October by the cone-bearing canopy foliage (Holthuijzen et al. 1987).

### 3.2.6 Monitoring the seed crop

The sequence and length of different components of the reproductive cycle must be understood when planning and executing seed production studies because different components are not the same in each species. For example, you need to know the length of the entire reproductive cycle of each species involved in the study, since this will determine when monitoring should begin. You must also know the timing of other critical events, such as pollination, fertilization, and periods of bud dormancy, to ensure you are at the right place at the right time.

For Douglas-fir, redcedar, spruces, true firs, and hemlocks, the development and maturation cycle takes about 16 months (Figure 3.1). For these species, male and female strobili appear in the spring, and seeds mature in the fall of the same year. In maple, alder, birch, Garry oak, and willows, seeds are also produced in the same year as the female flowers. However, in pines, complete development takes 26 months because fertilization is delayed for 1 year after pollination. In yellow-cedar, pollination and fertilization take place in the same growing season, but the total cycle usually lasts about 28 months. Under natural conditions, seed maturation is delayed by a period of dormancy until the following year (Figure 3.1); however, under favourable conditions in seed orchards, pollination, fertilization, and seed maturation can occur within the same year (El-Kassaby et al. 1991).

In conifers, male and female strobili appear on the same tree (with the exception of yew). However, the distribution of cones within the crown varies with the species (Table 3.2), and even within the same species, male and female cones may occur in different parts of the crown. In hardwoods, it may be necessary to identify male and female cones before monitoring, since dioecious hardwoods, such as Salix, Populus, and Fraxinus, bear male and female flowers on different trees.

### Monitoring pollen

Pollen abundance in the air during female receptivity is believed to be closely related to seed production. Estimates of pollen abundance can be used to formulate a relationship to (1) total seed production for the stand, and (2) total filled seeds per cone. In lodgepole pine, the number of male meristems produced on individual trees was correlated to the frequency of filled seeds on those trees (Smith et al. 1988). Weather data for previous and current year also can be incorporated to develop predictive models for seed yield. Possible relationships that can be studied are the amount of rain during flower initiation and temperature minima during critical stages of cone maturation (Stoehr and Painter 1995).

Many different kinds of pollen samplers have been used to assess pollen abundance. Each has its advantages and disadvantages, but often the choice depends on the financial resources available. The least expensive approach is a microscope slide placed on a flat surface or on the ground. The results from such a method may have little relationship to the actual densities experienced at the female cone level, but it may be possible to correlate the data with crown measurements.

Another inexpensive method used in Sweden and Finland is to trap old pollen strobili that fall to the ground to obtain estimates of pollen cone production (Leikola et al. 1982). At the other end of the spectrum

---

**Table 3.4 Rating of seed crops by number of filled seeds per hectare (Alexander et al. 1982)**

<table>
<thead>
<tr>
<th>Filled seeds per hectare</th>
<th>Seed crop rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 25 000</td>
<td>Failure</td>
</tr>
<tr>
<td>25 000–125 000</td>
<td>Poor</td>
</tr>
<tr>
<td>125 000–250 000</td>
<td>Fair</td>
</tr>
<tr>
<td>250 000–625 000</td>
<td>Good</td>
</tr>
<tr>
<td>625 000–1 250 000</td>
<td>Heavy</td>
</tr>
<tr>
<td>&gt; 1 250 000</td>
<td>Bumper</td>
</tr>
</tbody>
</table>
are automated air samplers that pull a known volume of air through the sampler so that air movement is dynamic. This differs from strip chart recorders, which are “passive” and depend on natural wind and air movement to adhere pollen to the “sticky” surface.

The abundance of pollen in conifer stands can be estimated using a 7-day pollen monitor (Webber and Painter 1994). Several monitors (three is good, depending upon the size of the plot) should be placed in the experimental sites 1 week before expected pollen shed and left in the field until the pollination period is completed. The monitor is mounted on a pole 4 m above the ground and always turns into the wind. The monitor consists of a permanent chart wrapped around a drum, which is rotated with a clock mechanism. The chart is made of mylar coated with petroleum jelly so that pollen will adhere; heating the petroleum jelly slightly creates a smooth, even coat. Since the drum completes one turn each week, pollen charts must be changed weekly. Pollen charts are assessed in the laboratory, where pollen densities, timing of dispersal, and pollen identification can be determined on a daily basis. Proper evaluation of pollen charts depends on the experience and expertise of an analyst familiar with pollen density patterns.

Sarvas (1962, 1968) used different types of pollen samplers to estimate pollen density in Scots pine (Pinus sylvestris) and Norway spruce (Picea abies), and was able to closely relate flowering to heat-sum calculations. He placed all his pollen samplers on towers at the height of the female flowers. Zasada et al. (1978) used this method for white spruce. Sarvas also used a small globe sampler to measure pollen rain density. The globe shape was chosen because it more closely resembled the shape of a female cone, and thus better approximated the pollen density patterns expected on a “cone-shaped” surface.

Another pollen trap described by Caron and Powell (1989a) consists of a glass rod (7 mm diameter × 10 cm long); one end is coated with a thin layer of white petroleum jelly to serve as the catching surface, the other end is tightly fitted through a rubber stopper into a hole on a wooden base. The square wooden base is grooved to slide into a holder, which can be set so that the edges are aligned to the four cardinal directions. Each trap holder is protected from rain by polyethylene film installed on a wire frame 15–20 cm above the base. A vial (25 mm diameter × 95 mm long) with a fitted rubber stopper is used to protect the catching surface from contamination before installation and after collection. The traps are collected and replaced daily.

Smith et al. (1988) sampled airborne pollen for density estimates of lodgepole pine at canopy level near the centres of two squares that made up a central rectangle surrounded by a 10 m bank. Air samples were taken at 20-minute intervals over 17 or 21 days with Kramer-Collins air samplers (Kramer et al. 1976). Pollen counts averaged over the 7 peak days were used to calculate relative pollen densities in the 127 stands chosen for study. Pollen cone production was estimated by counting the number of terminal meristems producing male strobili. Using binoculars, terminal meristems were counted for either the entire tree, or 100 or 200 meristems were counted on a portion of larger trees, and that portion was estimated as a percentage of the total surface area to provide a meritum total for the tree. Estimates ranged from 0 to 8000 male meristems per tree. To check the consistency of the technique, 131 trees were counted on consecutive days. Of the 114 counts that differed on the 2 days, the second day had the smaller count 52 times. The smaller count averaged 75% of the larger count for the 126 trees that had male meristems. Using a similar sight estimation of female cones on trees that were later cut down so that cones could be counted, Elliott (1974) and Smith (1981) found that their under- or overestimation deviated from the actual count by an average of 21%.

Pollen from different species can usually be identified microscopically (Figure 3.5). In a study of black spruce, the pollen catch was systematically examined (100-power magnification) on the four directional faces of each trap (Caron and Powell 1989a). Pollen identification to the species level (or at least genus) was accomplished by comparing pollen samples collected directly from trees with micrographs and species descriptions (Richard 1970; Adams and Morton 1972). In mixed stands comprised of species with similar-looking pollen, it is advisable to install additional pollen monitors in pure stands of the species located near to the study site. Pollen density records from mixed stands can then be compared to those from pure stands to determine the relative pollen abundance and time of pollination of different species (Stoehr and Painter 1995).
Monitoring seed cones

This section focuses on monitoring female conifer cones, although the same procedures could also be used for the flowers, fruits, and seeds of hardwood species.

To sample for cone production (either male or female cones) it is necessary to determine where the cones are produced. In western larch, most seed cones are produced on ascending branches or on recent terminal leaders within the upper third of the crown; most pollen cones are found on horizontal or descending branches within the lower two-thirds of the crown (Shearer and Schmidt 1987). In British Columbia considerable overlap of the seed and pollen cones occurs within the lower half of the crown (Owens and Molder 1979a, 1979b) (Table 3.2).

In species such as whitebark pine, cone scars can be monitored to estimate past cone crops (Morgan and Bunting 1992) (Figure 3.6). Morgan and Bunting chose a 90 m transect that contained 10 mature, cone-producing whitebark pine trees with crowns.

Figure 3.5 Scanning electron micrographs showing whole pollen and details of the exine. (a), Chamaecyparis nootkatensis (x1100); (b), Betula (x860); (c), Abies amabilis (x400); (d), Pinus contorta (x720); (e), Pseudotsuga menziesii (x360); (f), Tsuga heterophylla (x540). (Owens and Simpson 1986).
Similarly, in Douglas-fir, it is possible to trace pedicle remains to estimate previous cone production (Tappeiner 1969). In Abies, cone spindles remain on branches for several years after the cones have disintegrated; these also might be used to estimate previous production (J.C. Tappeiner, pers. comm., 1997).

In a study of ponderosa pine, four branches in the upper half of the crown were randomly selected and permanently marked for the presence of male and female flowers (strobili), conelets, and mature cones (Heidmann 1984). Flowers were counted in July of each year for 4 years. Because of the great number of male flowers on some branches (as many as 150 clusters per branch), an average was determined for a sample of 20 clusters, then multiplied by the number of clusters to obtain the total flower count for that branch. All female flowers were counted.

Fourteen western larch stands ranging in age from 46 to 100 years were monitored by Shearer and Carlson (1993) in Idaho, Montana, Oregon, and Washington. Using binoculars, they estimated the number of new seed cones in spring. Five trees with the greatest seed cone counts were climbed and the number of developing cones was estimated by counting the number of branches with seed cones, and new seed cones (living and dead) on six random branches (two from each third of the crown). The number of potential seed cones was estimated by multiplying the average number of cones per sample branch by the number of cone-bearing branches. Seed cone survival was estimated in August by counting the number of cones that matured on the six branches selected in the spring. Seed cone mortality was determined by subtracting surviving cones from the total cones counted at the first visit of the year. During the first visit, researchers marked 25 cones on the two trees bearing the most cones at each site. During subsequent visits they documented cone development, as well as the time and cause of cone damage. Dead cones were removed and the probable cause of death was identified.

Cone and seed analysis
Initially developed for southern pines, cone and seed analysis is an excellent procedure for identifying actual and potential seed production and the causes of seed loss in conifers. Bramlett et al. (1977) provide complete background and procedures.
Cone and seed analysis is based on calculating four critical ratios (efficiencies), which are used to identify the sources (stages) in which major losses occur:

- **Cone efficiency** = \( \frac{\text{number of cones harvested}}{\text{number of conelets initiated}} \)
- **Seed efficiency** = \( \frac{\text{number of filled seeds}}{\text{number of fertile sites}} \)
- **Extraction efficiency** = \( \frac{\text{extracted seeds per cone}}{\text{total filled seeds per cone}} \)
- **Germination efficiency** = \( \frac{\text{number of germinated seeds}}{\text{total filled seeds}} \)

The analysis requires the determination of:
- the potential number of seeds per cone;
- the total number of seeds per cone;
- the number of extracted seeds per cone;
- the number of filled seeds per cone; and
- the number of empty and insect-damaged seeds per cone.

Note that the number of filled seeds must be determined in addition to the total number of seeds. This is essential to reflect the actual seed production potential of the species.

Commercial services are available if you do not wish to perform your own cone and seed analyses (refer to Portlock [compiler] 1996).

Cone and seed analysis has been applied in British Columbia to analyze seed production of lodgepole pine and Douglas-fir (McAuley 1989a, 1989b). For the analysis, samples were randomly selected from five sacks among those filled that day. Random subsamples consisting of one cone per sack were placed in separate bags for subsequent analysis. Based on previous experience, a sample size of 40 Douglas-fir cones per orchard (45 cones for lodgepole pine) was considered to yield a reasonably precise estimate of single, orchard-level means. Samples for cone and seed analysis of western larch consisted of 20 cones collected from each of 20 individual trees per hectare (Stoehr and Painter 1995).

Hardwood trees could also be assessed using cone and seed analysis methods. Determining the cone and seed efficiencies would require some modifications since, in species such as *Salix* and *Populus*, catkins are comprised of capsules, each of which has several to many seeds. In *Alnus* and *Betula*, catkins are more like conifer strobili. In *Acer* and *Fraxinus*, fruits are paired or single samaras, respectively, each containing one seed per samara. Refer to Table 3.1 and to Section 3.3.1 for further information on hardwood fruit characteristics.

Whether the method is used for conifers or hardwoods, two cautions should be considered in conducting cone and seed analysis and extending the results to the species:
1. If at all possible, the cone analysis should be repeated during another good seed crop year.
2. If an unharvested stand can be found near the study plot, cones should also be collected from the unharvested stand for comparison.

**Filled seeds per cone**

Measurements of the number of filled seeds per cone are obtained primarily for predictive purposes. Numbers are used to plan the size of cone collections in a particular area, or to estimate the potential of a site for natural regeneration. Large samples are generally not needed. For example, in ponderosa pine only 20 closed cones from each lot were required to obtain good correlations between filled seeds per cone and kilograms of seeds per hectolitre of cones (Ready 1986).

Determining the total number of filled seeds per cone is time consuming, as it requires complete dissection of the cone. Special tools are needed as most cones are hard and woody. For these reasons many studies have attempted to relate the number of filled, sound seeds seen in the cone half-face to the total number of seeds in the cone (see Figure 3.7). Schmid et al. (1985) tested several sampling designs to determine the accuracy and precision of each design in estimating the mean numbers of filled seeds. They found that half-face counts on 20 cones (two cones from each of 10 trees) from a ponderosa pine stand estimated the filled-seed percentage for whole cones within ±10 units of the mean. Olsen and Silen (1975) multiplied the number of filled Douglas-fir seeds seen in the cone half-face by 4.5 for an estimate of the total seeds per cone. From each 7.6 L of undried cones, they cut 10 cones in half longitudinally,
counted the number of full seeds on one cut surface, then dried and extracted the cones to determine the total number of filled seeds.

Half-cone counts have been used extensively in British Columbia to determine whether developing cone crops are collectable. Recommended collection standards based on filled seeds in the cone half-face are given in Eremko et al. (1989) for Abies amabilis, Abies grandis, Abies lasiocarpa, Chamaecyparis nootkatensis, Larix occidentalis, Picea glauca, Picea mariana, Picea sitchensis, Pinus contorta, Pinus monticola, Pinus ponderosa, Pseudotsuga menziesii, Thuja plicata, and Tsuga heterophylla.

3.3 Determining Fruit and Seed Maturity and Quality

Understanding fruit and seed morphology is vital in designing and implementing a seed production study. A brief description of the seed-bearing structures of British Columbia tree species follows (summarized in Table 3.5). In addition, various cone and seed attributes (such as colour, weight, and length) can be used to indicate seed maturity. Assessment of seed maturity may be the objective of the study or may be important for obtaining the best-quality seeds for another study.

Because conifer and hardwood seeds can vary so greatly, the procedures for collecting, processing, and storing seeds of various species are discussed separately in Section 3.4 according to the characteristics of their fruits.

3.3.1 Description of conifer and hardwood fruits

The dry multiple fruit of a conifer is called a cone or strobilus (plural strobili). A female cone consists of a central axis supporting overlapping bracts, each of which subtends a scale bearing naked seeds. Gymnosperm, another term for conifer, means “naked fruit,” referring to the fact that conifer seeds are borne
The seeds of hardwoods are enclosed in protective fruits that vary considerably in size, colour, and structure. The seeds of bigleaf maple and Oregon ash are contained in dry winged fruits called samaras. In maple, two winged seeds are joined to form a V, but in ash, each fruit contains only a single winged seed. The fruits of red alder, birch, poplar, and willow are

### Table 3.5 Seed-bearing structures of trees occurring in British Columbia

<table>
<thead>
<tr>
<th>Fruit type</th>
<th>Definition</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>achene</td>
<td>Dry, indehiscent one-seeded fruit.</td>
<td>Betula</td>
</tr>
<tr>
<td>acorn</td>
<td>One-seeded fruit of oaks; consists of a cup-like base and the nut.</td>
<td>Quercus garryana</td>
</tr>
<tr>
<td>aril</td>
<td>Exterior covering or appendage that develops after fertilization as an outgrowth from the point of attachment of the ovule.</td>
<td>Taxus brevifolia</td>
</tr>
<tr>
<td>berry</td>
<td>Pulpy fruit developed from a single pistil and containing one or more immersed seeds, but no true stone.</td>
<td>Arbutus menziesii</td>
</tr>
<tr>
<td>capsule</td>
<td>Dry, many-seeded fruit composed of two or more fused carpels that split at maturity to release their seeds.</td>
<td>Populus, Salix</td>
</tr>
<tr>
<td>catkin</td>
<td>Spike-like inflorescence, usually pendulous, of unisexual flowers (either staminate or pistillate). Also used to describe the fruit. Compare strobile.</td>
<td>Alnus, Betula, Populus, Salix</td>
</tr>
<tr>
<td>cone</td>
<td>Dry multiple fruit of conifers. A female cone consists of a central axis supporting overlapping bracts, each of which subtends a scale bearing naked seeds. A male cone consists of a central axis supporting spirally arranged microsporophylls bearing pollen sacs that contain the pollen grains. Syn. strobilus.</td>
<td>all B.C. conifers, except Taxus</td>
</tr>
<tr>
<td>drape</td>
<td>Fleshy indehiscent fruit, usually one-seeded, containing a seed enclosed in a hard, bony endocarp (pericarp). Syn. stone fruit.</td>
<td>Cornus, Prunus</td>
</tr>
<tr>
<td>nut</td>
<td>Dry, indehiscent, one-seeded fruit with a hard wall.</td>
<td>Quercus garryana</td>
</tr>
<tr>
<td>pome</td>
<td>Many-seeded fruit of the apple family consisting of an enlarged fleshy receptacle surrounding the papery, fleshy pericarp.</td>
<td>Malus fusca</td>
</tr>
<tr>
<td>samara</td>
<td>Dry, indehiscent winged fruit; may be one- or two-seeded.</td>
<td>Acer (two-seeded), Fraxinus (one-seeded)</td>
</tr>
<tr>
<td>strobile</td>
<td>Spiky pistillate inflorescence or the resulting fruit; not a true strobilus. Syn. female catkin.</td>
<td>Alnus, Betula, Populus, Salix</td>
</tr>
<tr>
<td>strubilus</td>
<td>Male or female fruiting body of the gymnosperms.</td>
<td>all conifers, except Taxus</td>
</tr>
</tbody>
</table>

Notes:
carpel: simple pistil or single member of a compound pistil.
imperfect flower: flower which contains either, but not both, functional male or female parts.
indehiscent: refers to dry fruits that normally do not split open at maturity.
nutlet: nut-like fruit or seed, as in *Alnus* or *Betula*.
perfect flower: flower that contains both pistil and stamens.
pericarp: wall of a ripened ovary that is homogeneous in some genera and in others is comprised of three distinct layers: exocarp, mesocarp, and endocarp. Syn. fruit wall.
pistil (or pistillate): the female part of angiosperm flowers, containing the ovary, from which seeds develop.
staminate: referring to male angiosperm flowers, containing the stamens, from which pollen is produced.
stone: part of a drupe consisting of a seed enclosed in a hard, bony endocarp as in *Prunus* and *Cornus*.
catkins (or strobiles), which develop from the spike-like female flowers. The drooping catkins of poplar and willow comprise many capsules that split open at maturity to release many seeds per capsule. The catkins of birch break up at maturity to release the small winged nutlets. The female catkins of alder are woody cones; the cones contain oval nutlets that do not break up at maturity. The fruit of Garry oak is an acorn, consisting of a hard-coated nut in cup-like base.

Several hardwoods have fleshy fruits surrounding their seeds. The fleshy fruit of Pacific dogwood, bitter cherry, and cascara is a drupe, sometimes called a stone fruit. A drupe usually contains a single seed enclosed in a hard, bony ovary wall (the stone). The arbutus fruit is a berry, a pulpy fruit developed from a single pistil (female part of a flower) and containing one or more immersed seeds, but no true stone. The Pacific crab apple is a pome, a many-seeded fruit consisting of an enlarged fleshy receptacle surrounding a papery ovary wall.

3.3.2 Assessing embryo development
The most commonly used indicators of maturity in conifer seeds are cone and seed colour, degree of cone opening, condition of the megagametophyte, and length of the embryo (Edwards 1980; Shearer 1985; Eremko et al. 1989). Cones lose moisture as they mature, and cone colour usually changes from green to brown. In the field, specific gravity of the cones has been used to monitor maturation of Douglas-fir cones (Shearer 1985). The rate of maturation is influenced by the number of degree-days (Mosseler 1992), elevation (Shearer 1985), and latitude.

Conifer seeds should not be collected until embryos fill at least 90% of the embryo cavity (Figure 3.8). Although collection can begin when embryos fill 75% of the cavity, collecting seeds when embryos are more mature will result in better-quality seeds (Edwards 1980; Zasada 1988; Eremko et al. 1989).

Embryo development and size may be determined destructively or non-destructively. Non-destructive methods depend on an external visual assessment of the seeds. For example, with paper birch it is possible to distinguish viable well-filled seeds from non-germinable seeds by viewing the seeds with a dissecting microscope equipped with substage illumination (Bevington 1986). The small size of eastern white cedar (*Thuja occidentalis*) seeds makes it difficult to determine if the seeds are filled. Briand et al. (1992) therefore used swelling of the embryo area as a means of classifying developed from undeveloped seeds. Seed colour can also be used as a key to viability, and is discussed in more detail in Section 3.3.3.

Destructive methods of seed assessment include cutting seeds open to expose the embryo. This procedure allows for a greater variety of measurements, such as embryo length, embryo cavity length, and cotyledon length. Alternatively, you can germinate the seeds and determine the anatomical characteristics of the embryos. Cotyledon numbers of ponderosa pine were determined by germinating 20 seeds, and selecting 10 germinants for scoring (Ager and Stettler 1983).

See Sections 7.2.5 and 7.2.6 for quick tests and other viability tests.

3.3.3 Assessing seed colour
Colour is frequently used as an indicator of both cone and seed maturity. In some instances, the purpose of the study may be to assess seed colour as an indicator of seed maturity. In other instances, determining maturity (using colour) may be simply a tool to ensure that the best quality seeds are collected.

Seed colour is one of the more difficult indicators to quantify, as it relies on the subjective judgement of the observer, and cone and fruit colour can vary among different individuals of the population. Several instruments, such as Tristimulus colorimeters and video imaging systems (McGuire 1992), can be
used to quantify seed colour and reduce the subjectivity of colour readings.

Seed colour variation in ponderosa pine was quantified by constructing a 12-seed gradient with seeds from the entire collection (one seed from each of 12 trees) (Ager and Stettler 1983). Trees were then scored by comparing the adaxial (exposed) surface of five typical seeds from a given tree with the gradient. Mottled seeds were evaluated on overall shade. Although there were large colour variations among the populations studied, the seeds of a single tree were remarkably uniform when compared to seeds from different trees. This uniformity is probably a result of the high heritability and maternal control of seed morphology in pines (Kraus 1967).

Seed colour can be used as a key to the viability of willow and poplar. In willow the presence or absence of the embryo can be determined by the dark green of the cotyledons showing through the transparent seed coat.

3.3.4 Measuring cone and seed dimensions

Cone dimensions
Cone length can be measured using vernier calipers (0.05 mm precision) (Caron and Powell 1989a). Depending on the type of data presentation or data analysis, it may be convenient to group measurements of cone length into classes. Bergsten (1985) initially grouped cone length measurements of Scots pine into 18 classes (2.5 mm each) from 15.0 to 60.0 mm, but subsequently combined them into six length classes.

Temperature and humidity may affect some cone measurements. In western larch and subalpine larch, Carlson and Theroux (1993) measured cone length and diameter on both wet and dry cones, because moisture differentially influences their shape. They hydrated dry cones by placing them in a chamber at 100% humidity for 24 hours, then measured cone diameter at the midpoint along the longitudinal axis of the cone.

Cone measurements are sometimes used as stable taxonomic markers to distinguish between species of the same genera, and their hybrids. Carlson and Theroux (1993) measured the length and width of five scales and five bracts of western larch and subalpine larch, randomly selected from the middle one-third of each cone (measured when dry) to the nearest 0.01 mm.

Bract length was measured from the base to the tip of the pointed apex; width was measured at the widest point of the bract.

Large differences in cone morphology may be noted between stands and between years. Abnormal cone morphology may also be observed, for example, “forked” cones, proliferated cones (with needles formed at the apex), and combinations of male and female in the same cone. (See Zasada et al. 1978 for examples in white spruce.)

Seed dimensions
Measurements of seed size (length, width, and thickness) will depend on the anatomical characteristics of the seed (Figure 3.9). In ponderosa pine, Ager and Stettler (1983) defined seed length as the distance between the micropylar and basal ends, and width as the maximum distance across the seed perpendicular to the long axis. Length and width data were based on five seeds per tree.
In western larch and subalpine larch, Carlson and Theroux (1993) measured seed length, width, and thickness to the nearest 0.01 mm. Width and thickness were measured at the widest part of the seed, then each seed was sliced longitudinally. The thickness of the seed coat was measured to the nearest 0.01 mm midway between the base and apex of the seed. Sampling was done on 10 seeds randomly selected from each lot, as initial sampling estimates indicated that this sample size would enable standard errors within 20% of the mean with 95% confidence.

Briand et al. (1992) used a dissecting microscope equipped with an ocular micrometer to measure the small seeds of eastern white cedar (Thuja occidentalis). Seeds were positioned such that the micropylar end was facing up and the concave face of the seed was towards the viewer. The following measurements were determined to the nearest 0.1 mm: length and width of the seed and the embryo area, length of the right wing, and right wing width measured at the midpoint (Figure 3.10).

Extremely small seeds of Salix and Populus, which can be especially difficult (and tedious) to measure, can be graded by sifting them through a set of soil screens. Although this method is not as precise as using a micrometer, it is effective and less expensive (J. Zasada, pers. comm., 1996).

![Figure 3.10](image)

**Figure 3.10** Outline drawing of a typical seed of Thuja occidentalis (Briand et al. 1992) showing significant seed dimensions. LEA = length of embryo axis; W = width of entire seed; LRW = length right wing; WRW = width right wing; WEA = width embryo axis.

If X-ray equipment is available, seeds can be placed on celluloid film and exposed to X-rays (see Section 7.2.6). Once developed, the films can be placed on a microfiche viewer (of the type commonly used in libraries). Precise seed dimensions can be obtained by direct measurement of the projected images. Actual and projected dimensions can be compared to calculate an appropriate conversion factor. If the size and shape of seeds are suitable, an overhead light projector and 35 mm camera film can be used in a similar manner.

Anatomical measurements were made on white spruce seeds by cutting the seeds longitudinally and measuring the embryo length, embryo cavity length, and cotyledon length with a micrometer mounted in the eyepiece of a binocular microscope (Zasada 1988). When multiple embryos were present, embryo measurements were made on the dominant embryo. Samples consisted of 10 white spruce seeds taken from the central portion of four cones from each tree. In many conifers, seeds at the apical and basal portions of the cone are poorly developed (Bramlett et al. 1977).

### 3.3.5 Estimating seed weight and volume

Seed weight can be expressed as the fresh weight (fw) or dry weight (dw) of seeds. The expression used for seed weight will depend on the context in which it is used. International seed testing rules prefer the use of fresh seed weight (before drying in an oven), whereas ecologists more often use the dry weight of seeds. See Section 7.2.2 where fresh weight and dry weight are more thoroughly discussed.

Seed weights should be determined to at least two significant figures. The sample size required to estimate seed weight varies with the species and the variability of the crop. For example, two 10-seed replicates per tree were used by Ager and Stettler (1983) to determine the weight of ponderosa pine seeds. International standards for sample sizes for weight measurements may be found in International Seed Testing Association (1993) or the Association of Official Seed Analysts (1993). Seed weights of tree species occurring in British Columbia are listed in Table 3.6.

For serotinous cones of species such as jack pine and lodgepole pine, the volume of cones can be determined by immersing individual cones in a graduated cylinder containing water and a wetting agent (Rudolph et
al. 1986). A similar procedure has been used effectively for white spruce cones (Zasada et al. 1978).

### 3.4 Collecting and Processing Seeds

In many studies, seeds are an end product by which successful reproduction is assessed. Thus, efficient methods of collecting, extracting, and storing seeds must be known. The method selected for collecting and extracting seeds depends on the species being studied. Conifers generally require some effort to extract the seeds from the cone, and hardwood seeds are enclosed in a hard or fleshy fruit which must be removed to obtain the seeds.

Another factor affecting seed collection is the capacity of seeds for long-term storage. All conifer seeds and many hardwood seeds can retain viability for long periods if seed moisture content (mc) is reduced to low levels (5–10%) and the seeds are stored at subzero temperatures. Such seeds are called

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Seeds per gram</th>
<th>Scientific name</th>
<th>Seeds per gram</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gymnosperms</strong></td>
<td></td>
<td><strong>Gymnosperms</strong></td>
<td></td>
</tr>
<tr>
<td>Abies amabilis</td>
<td>25</td>
<td>Tsuga heterophylla</td>
<td>655</td>
</tr>
<tr>
<td>Abies grandis</td>
<td>50</td>
<td>Abies mertensiana</td>
<td>251</td>
</tr>
<tr>
<td>Abies lasiocarpa</td>
<td>85</td>
<td></td>
<td>132–458</td>
</tr>
<tr>
<td>Chamaecyparis nootkatensis</td>
<td>240</td>
<td></td>
<td>1468</td>
</tr>
<tr>
<td>Juniperus scopulorum</td>
<td>60</td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>Larix laricina</td>
<td>701</td>
<td>Alnus rubra</td>
<td>1468</td>
</tr>
<tr>
<td>Larix lyallii</td>
<td>313</td>
<td>Arbutus menziesii</td>
<td>570</td>
</tr>
<tr>
<td>Larix occidentalis</td>
<td>302</td>
<td>Betula papyrifera</td>
<td>3040</td>
</tr>
<tr>
<td>Picea engelmannii</td>
<td>300</td>
<td>Cornus nuttallii</td>
<td>10</td>
</tr>
<tr>
<td>Picea glauca</td>
<td>405</td>
<td>Fraxinus latifolia</td>
<td>18</td>
</tr>
<tr>
<td>Picea mariana</td>
<td>890</td>
<td>Malus fusca</td>
<td>119</td>
</tr>
<tr>
<td>Picea sitchensis</td>
<td>465</td>
<td>Populus balsamifera</td>
<td>3766</td>
</tr>
<tr>
<td>Pinus albicaulis</td>
<td>6</td>
<td>ssp. balsamifera</td>
<td>3583–3949</td>
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<td>Populus balsamifera</td>
<td>1652</td>
</tr>
<tr>
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<td>ssp. trichocarpa</td>
<td>1233–2070</td>
</tr>
<tr>
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<td>8353</td>
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<tr>
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<td>Prunus emarginata</td>
<td>15</td>
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<tr>
<td>Pinus monticola</td>
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<td>Quercus garryana</td>
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</tr>
<tr>
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<td>25</td>
<td>Rhamnus purshiana</td>
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</tr>
<tr>
<td>Pseudotsuga menziesii var. glauca</td>
<td>85</td>
<td>Salix amygdaloides</td>
<td>5720</td>
</tr>
<tr>
<td>Pseudotsuga menziesii var. menziesii</td>
<td>95</td>
<td>Salix discolor</td>
<td>no data available</td>
</tr>
<tr>
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<td>Salix exigua</td>
<td>22 000</td>
</tr>
<tr>
<td>Thuja plicata</td>
<td>915</td>
<td>Salix lucida ssp. lasiandra</td>
<td>25 000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Salix scouleriana</td>
<td>14 300</td>
</tr>
</tbody>
</table>

Sources: Stein et al. 1986; Wyckoff and Zasada [1998]; Zasada and Strong [1998]; Zasada et al. [1998].
orthodox in their storage behaviour. Some hardwood seeds do not store well, remaining viable only for several weeks up to 1–2 years. These seeds are called recalcitrant. They must be stored at relatively high moisture content (15–40%) and above zero temperatures, and may require other special handling.

3.4.1 Conifer seeds

Collecting conifer seeds

Conifer cones may be collected by climbing (Yeatman and Nieman 1978) or felling trees. Many aerial cone collecting techniques are also available (Camenzind 1990). Aerial methods are much more efficient, especially for species that produce cones in the upper crown, but collection costs are much higher since the use of a helicopter is required. Advantages and disadvantages of various cone collection methods may be found in Camenzind (1990). The choice of method for specific cone collection projects depends both on the crop and the techniques available. Factors to consider include species, crop size, quantity of cones to be collected, site characteristics, the capabilities of each harvesting technique, safety, efficiency, and cost.

For relatively small trees, and where conditions permit, cones and fruits can be collected using a fruit picker with a hydraulic lift. If cone-bearing regions are clearly visible, branches can be shot down with a rifle. Occasionally, cones may be collected from squirrel caches, but it is not recommended because the seeds may be infected with moulds and other pathogens (Sutherland et al. 1987).

Collecting Pacific yew and Rocky Mountain juniper seeds requires strategies different from most other British Columbia conifers. Both Pacific yew and Rocky Mountain juniper are dioecious and bear their fleshy fruits only on female trees.

The fruit of Pacific yew, which ripens in late summer or autumn, consists of a red, fleshy, cup-like aril bearing a single hard seed. To prevent losses to birds, yew fruits should be picked from the branches by hand as soon as they are ripe (Rudolf 1974).

The scales of the female flowers of Rocky Mountain juniper become fleshy and fuse to form small, indehiscent strobili commonly called “berries.” Immature berries are green; ripe berries are blue and covered with a white, waxy bloom. The fruit coat of Rocky Mountain juniper is thin and resinous. Juniper berries are usually collected in the fall by stripping or picking by hand directly into bags or baskets, or by shaking or flailing the fruits from the plant onto a canvas spread on the ground (Johnsen and Alexander 1974).

With all collection methods, safety precautions must be rigorously maintained. Safety belts and straps must be checked at least twice each day. Tools such as pruning poles and cone rakes should not be carried while the tree is being climbed. For aerial collections, the helicopter company must be certified, and the pilots appropriately qualified. Aerial collection operations in British Columbia are subject to Workers’ Compensation Board regulations; make sure that you have access to current regulations appropriate for the area, and confer with persons experienced in cone collection operations.

Extracting conifer seeds

Serotinous cones such as black spruce may require a period of high temperature to open the cone scales, and sometimes may need multiple extraction cycles, as for example, the procedure used by Haavisto et al. (1988):

1. soak cones in lukewarm water for 2 hours,
2. oven-dry cones at 40°C for 20–22 hours, and
3. tumble cones in a revolving screened drum for 30 minutes.

Using this procedure, an average of eight seeds per cone still remained after the 16th cycle (average seeds/cone = 85).

Note that the application of this procedure and the ones that follow will depend on the degree of serotiny of black spruce cones (see Section 3.5.1).

Mosseler (1992) used only two seed-extraction cycles (SEC) to remove most of the seeds from black spruce cones. The first SEC consisted of oven drying at 50°C for 24 hours. A second SEC was conducted after a 1-hour water soaking treatment, which was followed by drying at 50°C for 24 hours. Few seeds remained in the cones following this extraction procedure, and no further attempt was made to retrieve the remaining seeds. Seeds were counted with an electronic counter and were judged to be filled if they sank in 95% ethanol. Verification of ethanol separation was made by cutting sample seeds from the filled seed fraction, and crushing seeds from the empty fraction.
Although multiple extraction cycles were also used, the method used by Caron and Powell (1989a) differs significantly from the previous two, in that the black spruce seeds are not heated during extraction. Instead, after the cones were shaken individually in a covered jar to dislodge seeds, the remaining seeds were extracted with forceps. This shaking and seed-extraction step was repeated two or three times until all seeds were extracted. Cone scales were separated into three general categories (basal, central, and apical) before being counted. Central scales, which spread apart considerably on cone drying to permit easy release of seeds, were considered potentially seed-bearing (fertile). The extracted seeds were separated into filled and empty seeds by alcohol flotation (95% ethanol) after dewinging. X-ray analysis (see Section 7.2.6) indicated that 98.6% of the seeds that sank contained well-developed megagametophyte tissue and a fully developed embryo, whereas 92.6% of those that floated were empty or had a rudimentary embryo. Empty and filled seeds were counted and weighed to the nearest 0.1 mg. Cones (with seed wings) were dried in a forced-draft oven at 100°C for 48 hours and weighed to the nearest 0.1 mg.

In jack pine, which is a predominantly serotinous species, individual cones were dipped in boiling water for up to 30 seconds to break the resinous bonds between cone scales (Rudolph et al. 1986). The cones were dried in a circulating oven at 55°C until they were fully open, after which the cone scales were removed and the seeds were extracted by hand.

Seeds of most conifers (Douglas-fir, larch, western redcedar, western hemlock, etc.) are obtained by drying cones to open them, shaking out the seeds, separating the seeds from cone scales and debris, then loosening the seed wings, and finally separating clean full seeds from wings, dust, empty seeds, and other small particles. It may be advantageous to run closed cones over sorting tables or screens to remove foliage and debris before the cones open. On freshly picked cones of many species (e.g., Abies), pitch is soft and sticky. Chunks of pitch that become attached to extracted seeds may be extremely difficult to remove. Therefore, true firs should not be heated, but left under cool, dry conditions on trays to disintegrate naturally. Most other conifer species require only good ventilation and slight heating for several days to open the cones. Small lots of cones can be dried by improvised means in a well-ventilated laboratory oven with a circulating fan, over a hot-air register or radiator, or similar location.

Cones should be shaken or slowly tumbled to extract the seeds from the opened cones. Small-lot collections of seeds can be efficiently extracted using a multiple compartment tumbler-drier (Leadem and Edwards 1984). Although some wings are loosened during tumbling and preliminary cleaning, many conifer seeds must be dewinged. Wings of most pines and spruces separate readily from their seeds; the wings are hygroscopic, so slight misting can facilitate their removal. For Douglas-fir, larch, and true firs, wings cannot be removed from western redcedar or yellow-cedar without damaging the seeds.

Wings can be removed from small quantities of seeds by rubbing the seeds between the hands or against a screen or roughened surface. The same principle is employed for larger quantities by gently tumbling dry or wetted seeds in a rotating container such as a cement mixer. Loosened wings, small particles, and dust are removed from good seed in final cleaning. Small lots may be effectively cleaned using a laboratory aspirator (Edwards 1979) or by flotation in water.

The seeds of Pacific yew may be extracted by macerating the fleshy “berries” in water and floating off the pulp and empty seed (Rudolf 1974). Alternatively, the fruits can be soaked for 4–5 days in warm water, then rubbed over screens and washed thoroughly to float off light seeds. The viability of yew seeds can be maintained for 5–6 years if, just after extraction, they are dried at room temperatures for 1–2 weeks, and then stored in sealed containers at 2–5°C.

After twigs, leaves, and other debris have been removed with a fanning mill (air separation combined with screens), Rocky Mountain juniper seeds can be extracted by running the fruit through a macerator with water and floating away the pulp and empty seeds (Johnsen and Alexander 1974). Dried fruits should be soaked in water for several hours before macerating. Seeds should then be dried to less than 10% moisture content (mc) and stored between –6 and +5°C.

For additional information on conifer seed collection, processing, testing, and storage, refer to Stein et al. (1974), Edwards (1982), Eremko et al. (1989), and Leadem et al. (1990).
3.4.2 Hardwood seeds

Hardwoods are more variable than conifers in the time of flowering, seed maturation and dispersal, the type of seed-bearing structures (fruits), and the number of seeds per fruit (Tables 3.1, 3.2, 3.5). Many hardwoods are dioecious; in species such as ash, aspen, willow, and cottonwood, seeds are only produced on female trees. Since the fruits of species such as maple or ash contain only one seed, collection of hardwood seeds may be more labour intensive. For convenience of discussion, the maturation, collection, and processing of hardwood seeds is discussed by fruit type.

**Samaras (Acer, Fraxinus)**

Bigleaf maple seeds are double samaras, which turn from green to reddish brown when ripe. The pericarp has a dry, wrinkled appearance when fully mature, and the surface is covered with dense, reddish-brown pubescence. Within the pericarp is an embryo with associated seed coats, but there is no endosperm. Seed collection may begin when the Acer samaras are fully ripened and the wing and pericarp have turned tan or brown (Zasada and Strong [1998]). Acer seeds may be picked from standing trees or collected by shaking or whipping the trees and collecting the samaras on sheets of canvas or plastic spread on the ground. Samaras may also be collected from trees recently felled in logging operations, and sometimes gathered from the surface of water in pools or streams.

Bigleaf maple seeds should be collected before the fall rains. Once the fall rains start, seed moisture content (mc) may increase from 7 to 35% (dry weight basis) to as high as 50%. If bigleaf maple seeds remain attached to the tree, they may germinate (Zasada 1991). Moisture also affects the longevity of bigleaf maple seeds, which apparently can exhibit either orthodox or recalcitrant seed properties (Zasada et al. 1990; J. Zasada, unpublished data). The significance of collecting before or after the start of fall rains is that bigleaf maple seeds with low mc behave more like orthodox seeds, while seeds collected at high mc have characteristics similar to recalcitrant seeds. The pubescent pericarp may play an important role in the moisture content of the samaras.

Ash fruits occur in clusters of one-seeded samaras, and are collected in fall when their colour has faded from green to yellow or brown (Bonner 1974).

Another good index of maturity is the presence of a firm, crisp, white, fully elongated seed within the samara. The clusters can be picked by hand or with pruners and seed hooks. Fully dried samaras may be shaken or whipped from branches of standing trees onto sheets spread on the ground.

After collection, leaves and other debris can be removed by hand-stripping, screening, or using a fanning mill. Since the pubescence on the pericarp can be very irritating to the nose and skin, a face mask and rubber gloves should be used when working for extended periods with bigleaf maple seeds. Maple seeds generally are not extracted from the samaras following collection. However, dewinging reduces weight and bulk for storage, since wings account for about 15–20% of samara weight (Zasada and Strong [1998]). Empty samaras can be removed readily on a gravity table.

**Fraxinus** samaras should be spread in shallow layers for complete drying, especially when collected early (Bonner 1974). Dried clusters may be broken apart by hand, by flailing sacks of clusters, or by running fruits through a macerator dry. Stems and other debris can then be removed by fanning or with air-screen cleaners.

**Catkins (Alnus, Betula, Populus, Salix)**

Birch catkins should be collected while strobiles are still green enough to hold together, or immediately after a rain to keep them from shattering (Brinkman 1974a). In *Populus* and *Salix*, catkins should be collected as close to the time of seed dispersal as possible (Wyckoff and Zasada [1998]; Zasada et al. [1998]). Timing of collection can be based on catkin colour (which changes from green to yellow or yellow-brown) and the condition of the capsule. It is often best to wait until a few capsules start to split (Figure 3.11, stage b) and then collect catkins from the plant, since this usually results in the most rapid opening and efficient seed extraction. Note that insect-damaged capsules may appear to be dispersing seeds, but are often still immature. Once capsules begin to open, the rate of seed dispersal is determined by weather conditions; under warm, dry, windy conditions all seeds may be dispersed within a few days.

If only limited numbers of seeds are needed, branches with attached, immature catkins of *Populus*...
and *Salix* can be collected and ripened in a greenhouse or controlled environment (Wyckoff and Zasada [1998]; Zasada et al. [1998]). Catkins must be handled carefully after they have been removed from the tree. During transport catkins should be loosely packed in paper bags to allow for drying. Catkins placed in a warm dry spot will open in a few days, and seeds can be collected as the capsules open.

Since alder catkins do not disintegrate at maturity, they may be collected from standing or recently felled trees as soon as the bracts (scales) start to separate on the earliest-ripening strobiles.

After collection, catkins from *Betula papyrifera* can be air-dried on newspapers at room temperature (20–25°C), and the achenes separated from catkin bracts using a series of standard sieves, or with an air-driven seed blower (Bevington 1986). Seed samples can then be stored dry in sealed containers at -23°C until used.

*Populus* catkins should be spread out in thin layers in pans or on screens at room temperature (Wyckoff and Zasada [1998]). Seeds will be shed in 1–5 days, depending on the ripeness of the catkin. Seeds can be extracted from the catkins with a shop-type vacuum cleaner with a clean cloth bag substituted for the dust bag. *Populus* seeds can be freed from their cotton by tumbling the seeds in a rotating drum or a stream of relatively high-pressure air. For small quantities of seeds, the uncleaned seeds can be placed between two soil sieves and a high velocity air stream applied to tumble the seeds in the container. Seeds should be extracted and placed in subfreezing storage (-5 to -24°C) as soon as possible, since seeds stored at 0–5°C lose viability quickly. Storage with a desiccant appears to provide long-term benefit for *Populus* seeds (Wyckoff and Zasada [1998]).

*Salix* catkins should not be left at ambient temperatures, and seeds should be extracted and stored.

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**Figure 3.11** *Salix* capsules at various stages of opening (a–e) and the dispersal unit at various stages (f,g) (Zasada et al. [1998]). (f) shows hairs while still in the capsule; (g) shows hairs fully deployed and separating from the seed. Seeds should be collected when capsules start to split (b).
at low temperatures as soon as possible (Simak 1980). The seeds should be separated from the cotton to reduce bulk, and because storage with the cotton may reduce viability (Simak 1980). To clean small- to medium-sized lots, place catkins in a single layer in screen-covered boxes in a relatively warm, dry area (20–25°C, 25–35% relative humidity), with good air circulation (Zasada et al. [1998]). If capsules are beginning to open when collected, opening will be completed in 2–3 days. The seeds separate easily from the cotton if the catkins and the cotton containing the seeds are placed in a container, so the material can be blown in an air stream or tumbled in a cement mixer. Seeds can be separated from coarser and finer residue by passing them through a screen or sieve. At this time seed mc should be close to the 6–10% recommended for storage (Simak 1980). To maintain maximum viability, seeds should be placed in sealed containers and stored between -5 and -40°C.

Note that the seed quality of both Salix and Populus can be graded to a certain degree by passing seeds through a nest of soil sieves. In general, the largest and best seeds will be found on larger sieve openings (J. Zasada, pers. comm., 1996).

Alder strobiles will open after being exposed in drying racks in a well-ventilated room for several weeks at ambient air temperature (Schopmeyer 1974). They can be opened in a shorter time by drying them in a kiln at 27–38°C. Most seeds will fall out of the strobiles during the drying process; however, the remaining seeds may be extracted by shaking or tumbling if necessary.

**Nuts (Quercus garryana)**

Garry oak acorns are brown when they ripen in late summer and early fall; they may be collected from the ground, or flailed or shaken from branches onto canvas or plastic sheets (Olson 1974). Garry oak belongs to the white oak group, which is characterized by seeds with little or no dormancy, so acorns should be collected soon after they have fallen to retard early germination.

The only processing required before storing or sowing Garry oak acorns is removal of loose cups, twigs, and other debris (Olson 1974). However, the proportion of sound seeds can be increased by removing defective, hollow, and partially consumed acorns, either by flotation or by hand. To retain viability, acorns should be kept under moist, cold conditions. As a member of the white oak group, Garry oak exhibits recalcitrant storage behaviour (Section 3.4), so the mc must not drop below 30–50%.

**Drupe (Cornus, Prunus, Rhamnus)**

Dogwood fruits are ovoid drupes which ripen in fall. To reduce losses to birds, fruit should be collected as soon as ripe by stripping or shaking from the branches. Ladders may be useful for collecting fruit from taller trees (Brinkman 1974a, 1974b).

Bitter cherry fruits should be collected in late summer or early fall when fully mature and dark red. Fruits are collected by hand-stripping, or by spreading sheets of suitable material under trees to catch the natural fall or fruits shaken off the trees (Grisez 1974). Fruit may be carried in bags, but boxes or baskets provide better protection against bruising and spoilage.

Cascara fruits should be picked in late summer or fall. The fruits are relished by birds so they should be harvested about 2 weeks before they are fully ripe (Hubbard 1974).

To extract seeds of fleshy fruits, most species can be macerated in a blender. Maceration can be facilitated by softening fruits for 3–7 days in running water (or with daily water changes). The mixture is then placed in water to separate the pulp and empty seeds from the good seeds by flotation. Seeds are thoroughly air dried and placed in sealed containers for storage at 2–5°C.

Dogwood stones can be sown without extracting them from the fruit, but seeds to be stored usually are cleaned to reduce bulk (Brinkman 1974b). If fruits cannot be cleaned soon after collection, they should be spread in shallow layers to prevent excessive heating, although slight fermentation may facilitate removal of the pulp. The stones can be extracted by macerating the fruit in water and allowing the pulp and empty stones to float away. Clean, air-dried stones may be stored in sealed containers at 2–5°C.

For bitter cherry it is usually desirable to clean seeds of all pulp and juice (Grisez 1974). Cleaning is done by macerators with water to float off or screen out the pulp. Small quantities may be cleaned by soaking and rubbing over a screen. Fermentation has been used to soften fruit, but germination may be severely reduced if seeds are allowed to become too warm or to ferment too long.
Cascara fruits can be allowed to decay for a few days to soften the pericarp, but usually fruits are run through a macerator with water soon after collecting, then the pulp is skimmed off (Hubbard 1974).

**Berries (Arbutus menziesii)**
The fruit of arbutus is a berry with a thin, rough, granular skin, which is bright red or orange red when ripe. Berries can be collected from standing trees from October to December (Roy 1974).

Arbutus berries can be dried at room temperature or seeds can be separated from the pulp immediately after being collected. Fresh or dried fruit can be soaked in water in a warm place to soften the pulp. Fruits then can be macerated and the seeds separated from the pulp by flotation. Seeds or uncleaned berries should be thoroughly dried, then stored in airtight containers at 2–5°C (Roy 1974).

**Pomes (Malus fusca)**
The pomes of Pacific crab apple are yellowish to reddish when they ripen in late fall. Ripe crab apples may be collected either by picking the fruit from the tree or by gathering fallen fruit from the ground (Crossley 1974).

Pacific crab apple seeds may be extracted by putting the fruits through a macerator with water, floating off the pulp, and screening out the seeds. Seeds should be dried to less than 10% mc and stored at 2–5°C (Crossley 1974).

### 3.5 Assessing Factors that Reduce Seed Yields

Seed yields are sometimes lower than expected or predicted and we must identify when or why these losses occur, either to verify the value of predictive equations or to prevent future losses. In this section, we examine the effects of serotiny and predation on seed yields. Seed crop losses may occur due to environmental factors, disease, or animal predation, and can be analyzed using life tables (Figure 3.12). Life tables quantify the magnitude and sources of loss and are helpful in interpreting seed crop failure.

<table>
<thead>
<tr>
<th>Age interval (months)</th>
<th>Number cones alive</th>
<th>Mortality factors</th>
<th>Number dying</th>
<th>Percent mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–1</td>
<td>1182</td>
<td>C. pinus pinus</td>
<td>37</td>
<td>3.13</td>
</tr>
<tr>
<td></td>
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<td>Abortion</td>
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<tr>
<td></td>
<td></td>
<td>Missing</td>
<td>1</td>
<td>0.08</td>
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<td>Unknown insects</td>
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<td></td>
<td>76</td>
<td>6.42</td>
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<td>1–2</td>
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<td></td>
<td>Shoot borer</td>
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<td></td>
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<td>Conelets remaining</td>
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<td>Total mortality</td>
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<td>20.54</td>
</tr>
</tbody>
</table>

**Figure 3.12** Partial life table for 1981 jack pine conelet crop, Oneida County, Wisconsin (adapted from Rauf et al. 1985). Life tables quantify the magnitude and sources of loss and are helpful in interpreting seed crop failure.
3.5.1 Assessing serotiny

Some conifers do not shed their seeds when they mature in the fall, and instead may retain their seeds in the cones for several years. This is termed serotiny, but is sometimes called canopy banking (as opposed to seed banking in the soil). Serotinous species retain their seeds in tightly closed cones until high temperatures (such as those achieved in a forest fire) open the cones. The degree of serotiny appears to depend on such factors as the frequency of fire, the local climate, and hybridization between interior populations that are predominantly serotinous and coastal populations that are not. Serotiny has great silvicultural significance because large quantities of seeds are potentially available for release after fires or harvesting.

In British Columbia, coastal lodgepole pine is primarily non-serotinous, whereas interior lodgepole pine usually bears serotinous cones (Eremko et al. 1989). In both varieties, cones remain on the trees for many years, but freshly ripened cones have the highest number of viable seeds. Jack pine are serotinous over most of their range, although southern sources tend to be non-serotinous. Black spruce cones are semi-serotinous; the cones remain on the tree and the seeds are viable for several years (Safford 1974), and sometimes as long as 15 years (J. Zasada, pers. comm., 1997).

To estimate the quantity and quality of seeds available for regeneration, it may be necessary to assess the age of serotinous cones. Eremko et al. (1989) provide photographic examples of lodgepole pine cones in different age classes, and recommend that, for lodgepole pine, only cones in classes I and II (i.e., less than 5 years old) be collected. The cones should be only partially weathered and completely closed.

Viability of seeds in serotinous cones of harvested trees can decline rapidly, and older cones present in the slash may have to be discounted as a source for natural regeneration of a site. Ackerman (1966) found that 3 years after logging there was a substantial decrease in the germination percentage of seeds. To conduct his study Ackerman devised scales to classify the degree of serotiny and degree of weathering of lodgepole pine cones present in logging slash:

<table>
<thead>
<tr>
<th>Classes of resin-bond rupture</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. fully open cone</td>
<td>scales free over 81–100% of cone surface</td>
</tr>
<tr>
<td>2. partly open</td>
<td>scales free over 21–80% of cone surface</td>
</tr>
<tr>
<td>3. closed</td>
<td>scales free over 0–20% of cone surface</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Classes of weathering as index of age</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–3 yr</td>
<td>no evidence of weathering</td>
</tr>
<tr>
<td>2–7 yr</td>
<td>weathered over 5–25% of surface</td>
</tr>
<tr>
<td>6–13 yr</td>
<td>weathered over 26–50% of surface</td>
</tr>
<tr>
<td>12–20 yr</td>
<td>weathered over 51–75% of surface</td>
</tr>
<tr>
<td>16+ yr</td>
<td>weathered over entire surface</td>
</tr>
</tbody>
</table>

3.5.2 Assessing predation

Seeds represent an excellent food source because of their stored reserves, thus mature seed crops are attractive to insects, birds, squirrels, or other animals. This section primarily describes predation of immature seeds (pre-dispersal); for a detailed discussion of the predation of mature seeds, see Section 5.

During excellent seed years there are usually more than enough seeds to support both animal predation and natural regeneration. In moderate years, however, predation can present a problem. Since predator populations usually lag a year or so behind abundant seed crops, a mast year is often followed by a poor year with higher predator populations. Insect and disease damage to seeds and cones may range from moderate to severe, and sometimes can result in the loss of an entire seed crop (Miller et al. 1984; Schmid et al. 1984). Depending on the type of insect or disease, the attack may occur any time from bud initiation to final seed development. Damage may result in cone or seed abortion or in partial or complete destruction of cones or seeds (Mattson 1978). Effects are sometimes indirect, for example, insects or disease may cause the premature opening of cones so that seeds are shed before they are fully developed.

Insect predation can alter cone crop phenology (Rauf et al. 1985) and seed dispersal, and may cause conelet and cone mortality. Seed losses due to insect predation can be determined by dissecting the cones and examining cone length, width, and the number of sound, hollow, and insect-damaged seeds (Schmid et al. 1984). The percentage of seeds damaged in each cone may vary, depending upon the insect species.

In areas where squirrel predation can have a major impact on natural seed production, it is advisable to
collect cones early, but only if seeds can be ripened sufficiently under artificial conditions. Hurly et al. (1987) found that intensive harvesting by red squirrels began in early September, and most caching occurred in late September and October. Early in this period most cones cut were eaten rather than cached. Caches are easily found; cones can be recovered from the caches within 4 weeks following the peak of caching behaviour.

More information on cone and seed insects is available in Hedlin (1974), Hedlin et al. (1980), Ruth (1980), and Ruth et al. (1982).

Microbial diseases may also be considered seed predation, and substantial cone and seed losses due to disease occur each year. For further information on cone and seed diseases of North American conifers, refer to Sutherland et al. (1987) and Ruth et al. (1982).

3.5.3 Using X-ray analysis to determine causes of loss

Seed X-rays are a quick and effective way to analyze seed production, but they depend upon the use of expensive X-ray equipment. If such equipment is available, X-radiography can provide non-destructive measurements of the number of filled, immature, and empty seeds, as well as the numbers of seeds which have been damaged or attacked by insects (Figure 3.13). In research studies, comparison of seeds to their X-ray images facilitates the efficient removal of empty seeds. For detailed procedures, see Section 7.2.6 and Leadem (1984).

3.6 Experimental Design

“Cheshire Puss,” she began, “would you tell me, please, which way I ought to go from here?”

“That depends a good deal on where you want to go to,” said the cat.

(Lewis Carroll “Alice in Wonderland”)

A study design is a plan for obtaining the maximum amount of information from available resources (Sit 1995). A good design should begin with clear, well-defined objectives. Three general objectives of natural seed production studies are:

- estimation (e.g., how many seeds per cone?)
- modelling (e.g., what is the relationship between seed production and stand, tree, and crown characteristics?)
- comparison (e.g., is seed morphology the same in cliff and swamp areas?)

3.6.1 Estimation studies

To estimate a parameter such as the average number of seeds per cone or the total number of seeds in a plot, proper sampling design must be considered to ensure that the estimates are unbiased. Many sampling designs can be used, such as simple random sampling, cluster sampling, stratified sampling, and multistage sampling. For detailed discussions on these and other sampling schemes, refer to Cochran (1977), Thompson (1992), and Buckland et al. (1993).

![Figure 3.13](image-url)  
**Figure 3.13** X-rays of tree seeds (Leadem 1996). X-rays are used to determine whether seeds are fully developed, damaged, or have been attacked by insects. (a) mature seed: c = cotyledon, m = megagametophyte, r = radicle, s = seed coat; (b) immature seed; (c) insect larva; (d) damaged seed.
Regardless of which sampling design is chosen, sampling should be done through a random mechanism. This will ensure that no systematic bias will be introduced to the data. Sometimes, due to convenience or convention, an investigator may subjectively select samples that are considered typical for the population of interest. These samples, called representative and judgement samples, are discouraged because they are subject to personal bias and their statistical properties are unknown. Systematic samples are often taken because of their ease of execution. However, they can be unreliable, especially when the sampling scheme coincides with an underlying pattern in the sampling population. It is best not to consider systematic samples for estimation.

To capture the variability in the population of interest, the sampling design must provide an adequate sample size. The sample size depends on the variability in the population, the accuracy desired, and the cost. You may want to consider stratifying the collection of samples (e.g., collecting from different levels of the crown of a tree) to reduce variation and better understand effects of position (vertical and horizontal). For estimation type studies, the sample size can be determined using confidence interval methods. See Section 3.1.3 for a discussion and an example of sample size determination methods using confidence intervals.

3.6.2 Modelling studies

To sample for modelling, the sampling guidelines discussed above should be followed. All variables involved in the model must be sampled from the same sampling points. For example, if you want to relate the number of seeds per cone with the number of exposed seeds in the cone half-face, then the total seeds per cone and the seeds in the half-face must be determined from the same cone.

To model a relationship, there must be enough data to capture the relationship between the variables. A general rule is to have at least 10 data points per parameter involved in the model. For example, a straight line model,

\[ Y = a + bX, \]

has two parameters, \( Y \)-intercept \( (a) \) and slope \( (b) \), and requires at least 20 data points. A logistic model,

\[ Y = \frac{a}{1 + e^{b-cX}}, \]

has three parameters \( (a, b, \text{and} \) c) and requires at least 30 data points.

The data collected should also cover the full range of interest. For example, suppose you want to model the relationship between cone production and accumulated growing degree-days \( (\text{GDD}) \). If you want to use the model to predict cone production for 600–1300 GDD, then the data you use in developing the model must span the range 600–1300 GDD. The resulting model would only be suitable for predictions within this range; extrapolations beyond the range would be unreliable.

In general, more data points are needed for complex relationships than for simple relationships.

3.6.3 Comparative studies

In contrast to sampling for estimation and modelling, comparative studies require an experimental design. In a comparative experiment, treatments are randomly assigned to a number of experimental units (the smallest collection of the experimental material to which a treatment is applied). If you wish to compare seed morphology in two different habitats (e.g., cliff and swamp), five sites each can be selected randomly from all cliffs and swamps within the population of interest. Within each site, 10 trees can be selected for cone measurement. In this example, a site is the experimental unit; a tree or a cone is a subsample.

Comparisons based on a single application of the treatments are unreliable because variations are expected between experimental materials. Differences between a cliff site and a swamp site could be due to differences in the habitat, or to natural variation from site to site, or both. The only way to distinguish the possible causes of variation is to replicate the treatments.

Replication of a treatment is an independent observation of the treatment. The number of replications is the number of experimental units to which a treatment is assigned. Replication should not be confused with subsamples, which are multiple measurements of a single treatment. In the cliff/swamp example, each treatment is replicated five times. The 10 trees within each site are subsamples. Pseudo-replication occurs when replication is claimed when in fact there is none. Pseudoreplication usually leads
to underestimation of the variability in the data. See Bergerud (1988) for additional discussion of pseudo-replication.

The number of replications necessary for a study depends on the variability in the data, the size of difference you wish to detect, the significance level desired, and the desired statistical power. Power analysis is the computation of statistical power for an experimental design, and should be carried out before the experiment to determine the amount of replication required. For more discussion on the use of power analysis for sample size determination, see Cohen (1977) and Nemec (1991).

Random assignment of the treatments to the experimental material is also essential to sound experimentation. Randomization assures that no systematic bias is introduced to the experiment, and the natural variation is approximately the same within each treatment group. Sometimes random assignment of the treatments to the experimental material is not possible. In the cliff/swamp example, it is not possible for the experimenter to assign a cliff or a swamp to a particular location; an area is a cliff or a swamp before the experiment is even conceived. In this case, to satisfy the randomization criteria, cliff and swamp sites must be randomly selected from all cliff and swamp sites within the population of interest. Subsamples for measurements must also be randomly chosen within each experimental unit.

The design of a comparative experiment depends largely on the treatments to be compared, the experimental material available, and the type of data to be collected. Common experimental designs employed in seed production studies include completely randomized design, factorial designs, and randomized block design. For discussions on these and other experimental designs, see Sit (1995).

It is vital that the sampling design and experimental design optimize all essential factors of the study. Researchers should discuss their designs and analysis plans with a statistician before implementing a study to ensure that all relevant factors have been considered.

3.7 Data Analysis

The success of an experiment requires both a well-designed plan and an appropriate analysis method, because the two are closely related. The method of analysis depends on the design plan, while the design plan is strongly influenced by the analysis method deemed to be the most suitable for the data. The analysis method should conform with the design of the study, the nature of the data, and the study objectives.

3.7.1 Estimation studies
If the objective of a study is sampling for estimation, then care must be taken to ensure that the formulae for computing mean, total, and standard error are appropriate for the chosen sampling scheme. A common mistake is to use formulae for simple random sampling design in more complex designs, which results in underestimation of the standard error of the estimate. That is, the estimate would appear more reliable than it really is. Nemec (1993) provides an example that illustrates the consequences of using simple random sampling formulae for data collected from cluster sampling.

3.7.2 Modelling studies
When the objective is to sample for modelling, then regression and correlation are typical analysis methods. Depending on the relationship between the variables of interest, linear or nonlinear regression may be required. If the goal is to determine the best set of variables for predicting a relationship, then stepwise regression can be used to systematically eliminate any unnecessary variables.

Regression assumes that the residuals (the difference between the observed data and the predicted values) are normally distributed, with a mean equal to zero and a constant standard deviation. The normal distribution of the residuals can be checked using a normal probability plot on the residuals. An apparently straight line indicates that the residuals are approximately normally distributed. Regression also assumes that the residuals are: a) independent of the values in the explanatory variables (x-variables), and b) have equal variance for all values of the explanatory variables. The independent and equal variance assumptions can be checked by plotting the residuals against the predicted values derived from the regression model. A random scatter of the points implies that both assumptions are satisfied.

Violation of the normality and equal variance assumptions sometimes can be corrected by
transforming the data using square root, natural log, or exponential functions. Transformation should not be done routinely without first checking the residuals. Keep in mind that the regression assumptions are for the residuals, not for the data. It is possible to have non-normal data, but normal residuals. A common mistake is to examine the data and apply transformation when the data are not normal or have unequal variance.

Regression is a robust procedure against slight departures from normality and equal variance when the data set is large. That is, with a large data set, you can still use regression on the data (without transformation) for slightly non-normal residuals. However, like most statistical procedures, regression is not robust against independence. That is, regression results are invalid if the residuals are dependent (e.g., when large residuals tend to associate with large x-values.) Provision for randomization during data collection will ensure that the data, and thus the residuals, are independent.

To assess the goodness of fit of a model to the data, the coefficient of determination, $r^2$ value, can be calculated. The coefficient of determination represents the proportion of variation in the data explained by the model. The higher the $r^2$ value, the more variation is accounted for by the model. The $r^2$ value is directly related to the number of explanatory variables in the model: the more explanatory variables there are, the higher the $r^2$ value. When comparing several regression models, it is more suitable to use the adjusted coefficient of determination ($r^2_{adj}$) which is $r^2$ modified by the number of explanatory variables in the model. A model with large $r^2_{adj}$ is favourable, that is, it explains most of the variation in the data with the minimum number of variables. Rawlings (1988) may be consulted for further information on regression analysis. See also Sit and Poulin-Costello (1994) for additional discussions on nonlinear regression.

If the objective is to assess the strength of the relationship between two variables, then correlation analysis can be used. There are two types of correlation: Pearson product-moment correlation coefficient, $r(p)$, and Spearman’s rank order correlation coefficient, $r(sp)$. The Pearson correlation assesses the linear relationship between two variables (see Figures 3.14a and b), and is based on the observed data. Spearman’s correlation assesses the monotone relationship between two variables, that is, whether the two variables have a strictly increasing (linearly or nonlinearly) or strictly decreasing relationship (see Figures 3.14c and d). Spearman’s correlation is based on the rank order of the data, with tied scores assigned the average of the scores that would have been assigned had no ties occurred.

A correlation coefficient must have a value between +1 and -1. A positive value implies that the two variables increase together; a negative value implies that one variable increases as the other variable decreases. A Pearson correlation coefficient near zero implies there is no linear relationship between the two variables, but the two variables may be related in a nonlinear way (see Figures 3.14c, d, and e). A Spearman’s correlation coefficient near zero implies that the two variables do not increase or decrease together, but they may be related in a curvilinear manner (Figure 3.14e).

![Figure 3.14](image)

**Figure 3.14** Correlation coefficients for hypothetical relationships. $r(p)$: Pearson product-moment correlation coefficient; $r(sp)$: Spearman’s rank order correlation coefficient.
3.7.3 Comparative studies

If the objective is to compare the effects of several treatments on seed production, then analytical methods should be used. The method chosen depends on the nature of the data and the design of the study. For continuous data such as seed weight, seed length, or seed width, methods such as the $t$-test and analysis of variance (ANOVA) $F$-test could be used for analysis. Both the $t$-test and the ANOVA $F$-test assume normally distributed residuals. This assumption can be checked by plotting the residuals in a normal probability plot (see Section 3.7.2). If the residuals are far from normal, then nonparametric procedures such as the Wilcoxon tests could be used. Refer to Sections 3.7, 4.5.2, 6.5, and 7.2.5 for discussions of ANOVA analysis. See Sit (1995) for a detailed discussion of ANOVA.

For discrete data such as seed crop rating or the number of full and empty seeds on a tree, contingency table tests (chi-square test, Sections 5.5 and 8.3.4) or log-linear models could be used. If data are collected on the same units over time and the objective is to assess trend, then repeated measures analysis methods should be considered. See Nemec (1996) for detailed discussions of repeated measures analysis.

3.8 Seed Production Case Studies

Six seed production case studies, taken from the literature, are summarized below. To highlight the design and analysis aspects, the studies are presented in point form. The cautions given at the end of each case emphasize the items that require special attention to ensure that study objectives are met.

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**CASE STUDY 1**: Estimating potential Engelmann spruce seed production on the Fraser Experimental Forest, Colorado (Alexander et al. 1986)

**Objectives**
- To predict the frequency of good seed crops.
- To relate seed production to stand, tree, and/or crown characters (sampling for modelling).

**Study Design**
1. The sampling was carried out over a long period of time (annually for 15 years).
2. Thirteen permanent sample plots with 10 seed traps were randomly located in each plot.
3. Seed trap contents were collected each fall and again the following spring.
4. Only filled seeds were counted; the response variable was the number of filled seeds per trap.

**Data Analysis**
1. The sample mean (by plot) was the best estimate of average number of filled seeds per trap.
2. ANOVA was used to test for location and year effects.
3. Seed counts were transformed ($\sqrt{x + \frac{3}{8}}$) to stabilize variance.
4. Regression was used to relate the number of filled seeds per trap and the total seedfall per trap.
5. Stepwise regression was used to select the best set of stand, tree, and/or crown measures for predicting seed production.
6. Transformation of the data may be considered to correct for heterogeneity of variance before regression; possible transformations are square root and natural log.

**Cautions**
- Sampling units should be randomly selected from all possible units.
- Seed traps should be randomly located within each sampling unit (sample plot).
- Seeds from several traps should not be bulked.
- If data are collected over a long period of time, check whether the model residuals are independent; and consider using time-series models or repeated measures (incorporating lag variables in the regression).
CASE STUDY 3: Cone size and seed yield in young *Picea mariana* trees (Caron and Powell 1989a)

**Objectives**
- To investigate the variation in cone size, seed yield per cone, and seed weight from cones collected in 4 plantations in three consecutive years.
- To determine the correlation between cone size, seed yield per cone, and seed weight.
- To examine the relationship between pollen abundance and filled seeds per cone.

**Study Design**
1. Five plantations (8, 10, 12, 14, and 16 years from seed in 1980), located in northwestern New Brunswick, were used in the study.
2. Two study areas were selected within each plantation; trees were randomly selected for measurement.
3. Responses included cone length, cone weight, total scales per cone, potential filled seeds per cone, total seeds per cone, total filled seeds per cone, seed efficiency, weight of 1000 filled seeds, and weight of 1000 empty seeds.

**Data Analysis**
1. Correlation was used to assess the relationships of the nine response measures.
2. Regression was used to relate the number of filled seeds per cone to the number of pollen cones per tree; logarithmic transformation was used on the response variables to correct for unequal variance.

**Cautions**
- Correlation can be used to assess the relationship between two variables, but Pearson correlation can assess only linear relationships. It is possible that two variables are nonlinearly related and the correlation coefficient is near zero.
- A variable that shows a high correlation to seed yield per cone may not be the best predictor for seed yield; another variable that is nonlinearly related with seed yield may be a better predictor.
- Always plot the data in a scatter plot.
CASE STUDY 4: Prediction equations for black spruce seed production and dispersal in northern Ontario (Payandeh and Haavisto 1982)

Objective
• To use nonlinear regression to relate the number of black spruce seeds per cone with cone age and crown class.

Study Design
1. Data on seed production (number of seeds per cone and cone age in years) were collected from black spruce in three crown classes: dominant, codominant, and intermediate.
2. Two sets of data on seed dispersal across the stripcuts were also available for modelling.

Data Analysis
1. A simple exponential decay function, \( Y = B_1 e^{-B_2 X} \), was fitted to the data to relate the total number of seeds per cone \( Y \) with cone age \( X \) for each crown class.
2. An inverse sigmoidal function, \( Y = B_0 - B_1 (1 - e^{-B_2 X}) B_3 \), was fitted to the data to relate seed viability \( Y \) with seed cone age \( X \).
3. An exponential decay-exponential model, \( Y = B_1 X_1^{B_2} e^{-B_3 X_2} + B_4 X_1^{B_5} e^{-B_6 X_2} \), was developed to relate estimated seedfall per hectare \( Y \) with strip width \( X_1 \) and distance from stand edge \( X_2 \).

Cautions
• Enough points are needed to cover the entire \( X \)-range.
• Know the form of the equation, and the derivatives with respect to the unknown parameters, and an estimate of the parameters (starting point for iteration).
• Models should be compared based on adjusted \( r^2 \).
• Do not extrapolate results from the fitted models beyond the range of the original data.

CASE STUDY 5: Estimating sound seeds per cone in white spruce (Fogal and Alemdag 1989)

Objectives
• To determine whether the number of filled seeds per cone half-face is a valid indicator of the total number of seeds per cone.
• To determine the relationship between the number of filled seeds per cone and cone length and diameter.
• To determine whether the relationship is the same across time and location.

Study Design
1. Cone data were collected from three white spruce plantations in 1982 and 1984.
2. Seed counts were made on 10 cones from each of 20 trees at each location.
3. Measurements included cone length and maximum diameter, number of sound seeds per section on one cone half-face, and number of sound seeds per cone.

Data Analysis
1. The mean and coefficient of variation were calculated for each of the four variables for each location and crop year.
2. ANOVA was used to compare locations and years based on the number of seeds per cone.
3. Scattergrams were prepared for each location and year to visually assess possible relationships between number of sound seeds per cone and number of sound seeds per section, cone length, and diameter.
4. Multiple regression was used to relate the number of sound seeds per cone with the following independent variables: number of sound seeds per section, cone length, and cone diameter. Eight models were fitted to the data.

Cautions
• Use adjusted \( r^2 \) to compare models, not \( r^2 \).
CASE STUDY 6: Consistency of cone production in individual red pine (*Pinus resinosa*) (Stiell 1988)

**Objectives**
- To compare production by stand and by individual trees at two dates (1970 and 1984).
- To relate cone production to stem diameter and subsequent diameter growth.

**Study Design**
1. Data were collected from an 18-year-old red pine plantation that was established as a spacing trial.
2. Permanent sample plots were established for each of six spacings.
3. Cone counts were made on mechanically selected, numbered trees in 1970 and 1984.

**Data Analysis**
1. Linear regression with square root transformation was used to relate crop size to tree size at both dates, and to relate 1984 crop size to 1970 crop size.
2. Potential cone production was approximated using the sum of both mature and aborted cones.
3. Relationship between 1970 crop size to 1970–1972 basal area growth was also analyzed.

**Caution**
- Use adjusted $r^2$ to compare models.