Published papers have been removed from appendix 1.

Ollerton, J. (1993). *Ecology of flowering and fruiting in Lotus corniculatus L.*. PhD thesis. Oxford Brookes University.

Ecology of flowering and fruiting in *Lotus corniculatus* L.

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Contents

Abstract

Lotus corniculatus L. (Leguminosae), is a perennial herb common throughout Britain. Its main pollinators are bumblebees (*Bombus* spp., Apidae: Hymenoptera). This is a study of the ecological factors which are important to flowering and fruiting in the species, and some of their evolutionary implications. The work was carried out at Wytham Estate, Oxfordshire, U.K., mainly in an ex-arable field (Upper Seeds) and a more established grassland (Lower Seeds Reserve).

The literature on self-incompatibility in *L. corniculatus* is reviewed; there are conflicting reports, but wild material is fundamentally self-incompatible.

Plants in Upper Seeds are larger than in Lower Seeds Reserve. Comparative data on soil nutrients in the two sites suggests that the cause is the persistence of phosphorus from inorganic fertiliser.

There is a positive, linear relationship between plant size, flower production and fruit production. The species regulates investment in flowers mainly at the level of the whole inflorescence, rather than altering number of flowers per inflorescence. Within individuals, there are no consistent trade-offs between number of fruit per infructescence, numbers of seeds per fruit and seed weight.

Weather patterns only partially explain the flowering phenology of *L. corniculatus*. Timing of first flowering and peak flowering are correlated but are variable between individuals, and between years for the same individuals. They are not correlated with flowering synchrony. An individual's flowering pattern does not consistently affect fruit-set; the overriding determinant of fruit production is plant size. Selection is therefore unlikely to be acting on flowering time in this species.

The production of large numbers of self-incompatible flowers does not seem to reduce fruit-set; pollinators do not visit enough flowers per foraging trip (perhaps because nectar production is low) for geitonogamy to become a problem.

Seed predation by larvae of a chalcid wasp, a weevil and a moth differs between individual plants, but not consistently so between years. Seed predation is not consistently correlated with plant size, mean flowers per inflorescence, number of seeds per fruit or seed size. There is no evidence for selection acting on these traits through seed predation. Partially predated seeds are often viable, which may have implications for seedling demography. Seed predation and flowering phenology are not definitively linked, strengthening the argument that flowering time is not adaptive in this species.

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My parents made all this possible; I owe them too much for thanks to be enough....

Finally, I want to express my gratitude to Susie and Ellen, who put up with so much....*p.t.o.*

This work is dedicated to Susie and Ellen,

who made it all worthwhile.

Australia, here we come...

Chapter 1: Introduction

1. Plant reproductive ecology in context

Plant reproductive ecology[1] is a relevant area of research at all spatial scales, beginning with individual plants; for example Stephenson's (1982) study of timing of outcrossing on a single tree of *Catalpa speciosa* (Bignoniaceae). "Patches" of individuals within a population were looked at by Rasmussen & Brødsgaard (1992) who studied inter-patch gene flow in *Lotus corniculatus* (Leguminosae). Population-level studies are probably the commonest, such as that of Molau *et al.* (1989) who assessed seed predation in *Bartsia alpina* (Scrophulariaceae). Community-level interactions are also frequently found, for example Feinsinger's (1978) study of tropical forest plants and their hummingbird pollinators. Studies at a global geographic scale are restricted to Kochmer & Handels' (1986) work on large-scale patterns of flowering phenology.

In the context of plant ecology as a whole, plant reproductive ecology can link up with studies of population demography, such as the effect of seed predation on population flux (Andersen, 1989); ecological physiology, for example, the net cost of nectar production (Southwick, 1984); plant-animal interactions, which includes the majority of pollination research; population genetics, including studies of gene flow and genetic variation (*e.g.* Rasmussen & Brødsgaard, 1992) and community processes (Feinsinger, 1987).

The range of studies categorised above should give an indication that, with respect to ecology *per se*, plant reproductive ecology provides a linking theme between plant and animal ecology.

Plant reproductive ecology has traditionally been a very descriptive branch of the biological sciences; Raven (1983) has argued that such a reliance on description, with little experimental data, has left the area of plant reproductive ecology "moribund". I would argue against this position. "Description" has an important role to play in any scientific endeavour; one cannot design experimental procedures

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[1] By "plant reproductive ecology" I am really referring to "angiosperm reproductive ecology"; although other groups of seed and non-seed plants have been studied from the point of view of their reproduction, it is work on angiosperms which has dominated the literature.

if one does not have a rough idea of what to expect; hypotheses can never be blind to what is already known. Thus, a descriptive approach is valuable not only for those areas of study which, as Gould (1990) has noted, are incapable of being probed by empirical means and must be tackled in ways normally reserved for historians; it is also a route by which inroads can be made into an area of study in the earliest stages of that field's development. This is what I believe happened in plant reproductive ecology prior to the last twenty years. Given the more sophisticated experimental and statistical techniques now available, as well as the opportunity to draw on the previous two centuries of accumulated work, plant reproductive ecology is at an exciting stage in its development. Rapid advances have been made, and continue to be published, in the areas of pollination ecology and biology, resource allocation, gender function and mate selection; witness the plethora of books that have surfaced in the last few years, for example Jones & Little (1983), Real (1983), Lovett Doust & Lovett Doust (1988), Barth (1991), Dafni (1992), Marshall & Grace (1992), Wyatt (1992).

The origin of the research described here lies partly in a wish to fill what I perceived as an important gap in our knowledge. From a number of perspectives *Lotus corniculatus* L. is a well studied plant species, and we know much about its genetics, physiology and reproductive biology, though with respect to the last area, most work has been done in an agricultural context. Little work has been done on the reproductive ecology of the species in natural populations; previously, studies such as Stephenson (1984) and Stephenson & Winsor (1986) had worked with artificially set-up populations, in countries where *L. corniculatus* is not native. Certainly, there were no studies of flowering phenology and patterns of fruit set, one of the main lines of the research described here. Thus, there was potential to address this situation, and to contribute work which would add significantly to our understanding of the ecology of one plant species. At the same time, I wished to provide insights into plant reproductive ecology which are of more general application.

2. The study species: birdsfoot trefoil (*Lotus corniculatus*)

2.1 Description of *Lotus corniculatus*

Birdsfoot trefoil (*Lotus corniculatus* L.), a member of the Leguminosae, is native to Britain and Europe, as well as northern and eastern Africa, central Asia, and (as var. *japonicus*) China, northern Korea and Japan (Jones & Turkington, 1986). Numerous varieties and sub-species have been described; it is an important forage crop in the U.S.A and Canada and a number of cultivars exist. The species is an iteroparous perennial with a deep tap root, growing mainly in grasslands and heaths, though it is also found in more disturbed habitats. The shoots usually die back over the winter. Grime *et al.* (1988) described its established strategy as "intermediate between stress-tolerator and C-S-R" *i.e.* the species occupies an unspecialised position within Grime's Competitor-Stress Tolerator-Ruderal ordination triangle. Regeneration is mostly by seeds. The yellow, zygomorphic flowers are borne in inflorescences of 1 to 9 (usually 1 to 5) florets. The fruit is a seed pod containing between 1 and 30+ brown, sometimes mottled, seeds. The pod is dehiscent, splitting longitudinally and catapulting the seeds to distances of over five metres (Rasmussen & Brødsgaard, 1992). A proportion of the seeds produced by an individual have a very persistent hard-coat dormancy. *Lotus corniculatus* is polymorphic for a number of traits; the best studied are the biosynthesis of anti-herbivore cyanide compounds [see the sequence of papers starting with Jones (1962) and culminating in Jones (1977)], and the variation in the colour of the keel petals (Jones *et al.*, 1986).

2.2 *Lotus corniculatus* as a suitable species to study.

As well as the reasons outlined in section 1, the decision to use *Lotus corniculatus* for this study was based on the following considerations.

1. The species is common, and a large population exists at the Wytham site. Discrete individuals are found (except where the plant is heavily grazed) and so the common problem of "what is a single plant", often associated with perennial herbaceous species, is negated.

2. It is well studied compared with many of our other native species, so there is a lot of background material to work on.

3. As a typical legume, *L. corniculatus* has seeds which are held within a pod until some time after maturation. This makes the fruits convenient for a study such as this. As a comparison, one can look at the situation with regard to Wild Basil [*Clinopodium vulgare* L. (Labiatae)], another common plant of calcareous grassland, and a species which was originally considered as a potential focus for this study. The fruits of this plant form as one-seeded nutlets, sitting at the bottom of the open, dry calyx. Consequently, *C. vulgare* sheds its seeds readily and it is difficult to determine exactly how many have been produced by a flower.

2.3 Pollination biology

Those studies of *Lotus corniculatus* which are relevant to this project will be reviewed here; much fuller accounts of the biology of *L. corniculatus* are given by Turkington & Franko (1980) and Jones & Turkington (1986).

Proctor & Yeo (1973) include *Lotus corniculatus* in their lists of British plants visited by wasps (Vespidae: Hymenoptera) and moths and butterflies (Lepidoptera). Although I have never seen wasps visit any of the plants on the Wytham site, lepidopteran visitors include the six-spot burnet moth (*Zygaena filipendulae*: Zygaenidae), the small skipper (*Thymelicus flavus*: Hesperiidae) and the common blue butterfly (*Polyommatus icarus*: Lycaenidae). The authors go on to cite Müller as stating that:

"Common Birdsfoot Trefoil (*Lotus corniculatus*) is pollinated by bees...*Lepidoptera* take the nectar without causing pollination."

Casual observation of common blue butterflies feeding on *L. corniculatus* at Wytham would seem to confirm this statement; butterflies visiting the flowers hold themselves above the wing petals and so appear not to contact the reproductive structures. The most common visitors to flowers of *L. corniculatus*, and "the only effective pollinators" according to Knuth (quoted in Turkington &

Franco, 1980) are large bumblebees (*Bombus* spp. Apidae: Hymenoptera). The bumblebees are smeared with pollen when they force apart the wing petals in their probing for nectar and pollen; subsequent visits to other plants will bring about pollination in the same manner.

As part of a longer investigation of keel-petal colour polymorphism Jones *et al.* (1986) looked at the possibility that differential pollinator behaviour was maintaining the polymorphism. This proved not to be the case; nor could the phenomenon be explained by differential effects of seed predators, nor of differences in maternal output between the morphs. The reason(s) for the existence of the polymorphism remain a mystery.

Gene flow between patches of *L. corniculatus c.* 5m to *c.* 20m in diameter was investigated by Rasmussen & Brødsgaard (1992). A comparison was made between apparent gene flow, inferred from pollinator behaviour and seed dispersal, and genetic variation deduced by a DNA restriction fragment length polymorphism (RFLP) study. They found that gene flow via the bumblebee *Bombus lapidarius* L. was concentrated within patches of plants, as the insects were foraging within patches far more often than flying between patches, but pollen carryover between plants was significant. They concluded that, although gene flow between the isolated patches was relatively rare, it was certainly important, and showed that long distance seed dispersal by *L. corniculatus* was a much rarer occurrence than long distance pollen dispersal. Thus, pollen gene transfer between the patches was significant enough to prevent any genetic differentiation of one patch relative to another. When they studied their RFLP patterns, however, genetic differentiation between patches was found to be much more significant than had been suggested by the pollinator behaviour and seed dispersal studies. This apparent discrepancy was thought to be the result of random genetic events, such as genetic drift and founder effects, as the population was a young colonising one.

In their ambitious study of bumble-bee flower usage, Fussell & Corbet (1992) ranked *Lotus corniculatus* fourth (out of twenty) in usage by "black-bodied red-tailed" *Bombus* spp. (principally *B. lapidarius*). The plant did not seem to be a species of choice with other *Bombus* spp.

2.4 Maternal investment.

A number of aspects of maternal investment in *Lotus corniculatus* have been looked at experimentally by A.G. Stephenson and colleagues. Stephenson (1984) came to the following conclusions.

1. Under the experimental conditions provided, fruit-set is limited by resources, not pollen; hand pollinated ramets produced no more fruit than did open pollinated controls, whilst seed set was least in partially defoliated plants, intermediate in control plants, and greatest in nutrient enriched plants. Number of seeds per fruit remained constant and did not seem to be a method by which *Lotus* regulated its investment. Interestingly, though total fruit-set did increase over the three treatments (partial defoliation, control and nutrient enriched) because of increasing numbers of flowers, there was a consistent mean proportional fruit-set of about 30% between treatments. In this study Stephenson used a cultivar of *L. corniculatus* (*personal communication*, 1992); in a commercial variety consistency of seed production may be a trait that has been selected for.

2. The number of flowers produced by individuals was also resource limited. Once again, flower production increased over partial defoliation, control and nutrient enrichment treatments. This appears to suggest that *Lotus corniculatus* can, over the growing period, regulate its flower numbers. Though regulation of flower number was at the level of both number of flowers per inflorescence and number of inflorescences, 73% of the significant increase was accounted for by inflorescence production. This shows that flower regulation is primarily a function of the numbers of inflorescences produced. Whether this is true of native plants, in natural populations, is one of the questions which will be addressed in my study.

Stephenson's work is informative, but can be criticised for the following reasons.

1. The plants used were part of a sown plot of *Lotus corniculatus*, and to what extent this can be considered a good model of what a "natural" population would be doing is debatable. For example, it is perfectly feasible that, under certain circumstances, *Lotus* may be pollen limited. What is the effect of this on the

plants? Also, the plants were cultivars, which may have affected the results in ways other than the consistent 30% fruit set.

2. The author makes a passing comment about the fact that his treatments are not manipulating the same resources, but leaves it at that; I believe it may be more important than this casual dismissal warrants. Partially defoliating one set of plants and adding NPK fertiliser to another are not manipulations of the same resources; one changes the levels of mineral nutrients, the other levels of carbohydrates (photosynthate). Also, removing photosynthetic tissue from a plant can have effects beyond simply a reduction in photosynthesis *eg.* leaves act as sinks for mineral nutrients, so removing some of them means that more resources (of one particular type) may be available for flowering.

3. No assessment of ramet size was made, neglecting size-dependent effects. It is not enough to assume that ramets, because they are of the same age and have been growing under the same conditions, are the same size; micro-edaphic factors are just one possible source of variation.

At the end of the paper, the author speculates that *Lotus corniculatus* may be a species which can selectively abort fruits. This is confirmed in a follow-up paper (Stephenson & Winsor, 1986) which specifically tests the hypothesis that *L. corniculatus* can regulate the quality of its offspring. The authors found the following.

1. Plants can selectively abort those fruits with fewer seeds in them.

2. Seedlings from pods with greater numbers of seeds were more vigorous, and as adults had greater reproductive outputs.

Two hypotheses were put forward as potential explanations of this phenomenon.

a. Self-pollinated fruits often have fewer seeds in them than crossed fruits because of partial incompatibility and also due to the greater proportion of lethal recessives being expressed, resulting in early seed abortion.

b. Greater numbers of pollen grains on the stigma will result in increased pollen-tube competition, which is related to adult vigour, so the fruits with greater seed numbers should have seeds of better quality.

From this evidence it appears that *L. corniculatus* selectively aborts fruit on the basis of seed genotype. However, *Lotus corniculatus* under native conditions is highly self-incompatible (see section 2.5), so how important this mechanism is in natural populations, and whether it is an artifact of cultivar selection, is not known.

2.5 Self-incompatibility in *Lotus corniculatus*

The degree of self-sterility of *L. corniculatus* is of particular importance to one aspect of this study; the extent to which large numbers of flowers result in reduced fruit-set (see Chapter 4). Whilst the species possesses a number of mechanisms which ensure that outcrossing is maximised, and selfing kept to a minimum, the situation is rather complex and not entirely understood. The presence of a stigmatic membrane, breached only when a pollinator damages the stigma surface, acts as a physical barrier to a plant's own pollen (Seaney & Henson, 1970). This probably explains the difficulty some workers have had in performing hand pollinations with the species (D.A. Jones, *personal communication*, 1991). During the course of the present study an attempt was made to track the fate of self- and out-cross-pollen tube growth using fluorescent microscopy [see Hawes (1988) for techniques]. No pollen from either source was ever seen to germinate and grow. Somatoplasmic sterility means that self-pollen has a lower germination rate, and that pollen-tube growth is slower, reducing their competitiveness (DeGrandi-Hoffman & Collison, 1982). These authors go on to state: "...self-pollen tubes often stop growing before reaching the micropyle, and hence rarely fertilise the ovules." This can be contrasted with Seavey & Bawa (1986) who, citing a number of workers, believe that: "In *Lotus corniculatus* self pollen tubes are as successful as foreign ones at reaching the base of the style and...are able to penetrate to the base of the ovary." Possible reasons for this discrepancy are discussed below. Dobrofsky & Grant (1980a) have found that biochemical changes occur in selfed ovules before fertilisation of the ovule, and are interpreted as part of the self-incompatibility system. If self-fertilisation does occur, post-zygotic controls mean that fruits with a high percentage of selfed seeds have a greater chance of being aborted than do fruits with mostly outcrossed seeds (Stephenson & Winsor, 1986). This may be linked to work done by Miri & Bubar (1966), who found that selfed ovules possessed incompatible proteins in the somatoplasm which resulted in those seed pods aborting.

Published results of self-incompatibility assessments are, at first sight, ambiguous (Table 1.1).

Table 1.1: Comparison of results of *L. corniculatus* self-compatibility studies.

Published study	**Percentage selfing**	**Notes**
Darwin (1876)	"Several covered-up plants produced only two empty pods, and not a single good seed".	
Silow (1931)[1]	<1	Spontaneous
	<10	Hand pollinated
Macdonald (1946)	0	Spontaneous
	5	Hand pollinated
	100	*Bombus* pollinated
Bubar (1958)[2]	6	
Wojciechowska (1963)[2]	7	
Seaney (1964)[3]	50	Seed-set = <1 per flower
Miri & Bubar (1966)[2]	0 - 91	Dependent upon variety
Spiss (1969)[2]	<1	
Ramnani (1979)[1]	0	
Dobrofsky & Grant (1980b)[2]	50	
Brødsgaard & Rasmussen (1990)	0 - 16	only 1 out of 371 pods set any seed

Data culled from the original literature, and from [1]Jones & Turkington (1986), [2]Seavey & Bawa (1986) and [3]Turkington & Franko (1980).

Apparently only Darwin (1876), Wojciechowska (1963), Ramnani (1979) and Brødsgaard & Rasmussen (1990) used wild material in their work; the other studies employed cultivars. Self-compatibility is a trait that can be selected for (Seaney, 1964) and the use of cultivars is one possible reason for the perceived discrepancies. My own experience with the plants at Wytham is that covered flowers and hand pollinated flowers never set fruit. D.A. Jones (*personal communication*, 1992) has used an experimental set-up involving caged plants, with bumble bees acting as pollen vectors. Crosses involving different plants were successful, whilst the same set-up using clones of individual plants produced no fruit.

It appears from this that the hairy body of a bumble bee is needed to breach the stigmatic membrane, but this is not enough to guarantee self-pollination; at least in natural populations, there are other mechanisms to prevent self-fertilisation.

3. The Wytham study site

Wytham Estate, Oxfordshire, U.K. is owned and managed by the University of Oxford. The history and current status of the calcareous grasslands within the Wytham estate has been documented by Gibson (1986). The majority of the work described in my study uses plants from two sub-populations at sites within the estate: Upper Seeds and Lower Seeds Reserve. Two other sites were employed additionally in 1991: The Quarry and Rough Common. A brief description of these sites follows; the area has been more fully described by Gibson (1986), who also provides a map of the Wytham Estate.

Upper Seeds

This site is an ex-arable field of approximately 10ha, situated at the top of Wytham Hill. The soils are very thin, overlying Jurassic corallian limestone. Upper Seeds was a permanent pasture from at least the late eighteenth century until 1960, apart from a period of cultivation during the Second World War. From 1960 to 1981 the site was in cultivation; the field was then abandoned and the final crop of winter wheat left in the ground (Brown *et al.*, 1990). The processes of plant and invertebrate colonisation have been monitored ever since and the area has been the subject of a number of studies (some on-going) which have looked at a variety of topics, including: the seed bank (Woodell & Steel, 1990); changes in floristic diversity in relation to sheep grazing and insect herbivory (Gibson *et al.*, 1987a,b; Brown *et al.*, 1988); mechanisms of insect diversity (Brown *et al.*, 1990); regeneration and demography of grassland plant species (Antrobus, 1992; Fowler, 1993).

Lower Seeds Reserve

Lower Seeds Reserve is an area of calcareous grassland to the east of Upper Seeds, separated from it by a narrow strip of woodland. The site is dominated by *Brachypodium pinnatum* (Gramineae). The soil here is deeper and the flora much more established, as the site has not been cultivated since at least 1960, and perhaps not since the war. Grazing is restricted to deer and rabbits, but is generally not heavy.

The Quarry

The Quarry is the site of old limestone workings, probably abandoned at the end of the nineteenth century. The grassland which has subsequently formed is on extremely thin soil, and rabbit and deer grazing is heavy.

Rough Common

This is another area of established grassland, close to The Quarry, though with deeper soil. The whole area comprises a matrix of more or less heavily rabbit and deer grazed areas.

4. The aims of the study

The broad aim of this study is an investigation of some of the ecological, and potentially evolutionary, factors which influence flower production and fruit-set in *Lotus corniculatus*. In reality the ecology and evolution of any species are closely linked; the evolutionary history of *L. corniculatus* will determine its present ecology, whilst its ecology may result in selection pressures acting on the species. This study of the reproductive ecology of *L. corniculatus* will be tackled primarily from the maternal point of view; in many ways this is the more tractable half of plant sexual reproduction.

This research can be broken down into a series of topics, and I will devote one chapter to each. Within each chapter a series of questions will be posed, and perhaps answered.

Chapter 2 is concerned with the occurrence of large individuals of *Lotus corniculatus*, and of other plant species, within the Upper Seeds site. What I hope to answer is: Why are the colonising plants in Upper Seeds so much larger than the established plants in Lower Seeds Reserve and other areas of established grassland?

Chapter 3 examines how *Lotus corniculatus* allocates its resources to reproduction over time, and between the different components of reproductive output, and whether factors such as individual plant size and seed predation can affect this allocation. The questions which will be asked are: does *L. corniculatus* control its floral investment over time at the level of the individual flower or the whole inflorescence? How does the size of individual plants affect reproductive output in terms of flower and fruit production, seed size, and other maternal components? Do these factors vary between years? Can pre-dispersal seed predators affect resource allocation to undamaged seeds by their attacking other seeds within a fruit? Do resource trade-offs exist between the different components of maternal investment?

Chapter 4 is concerned with the flowering phenology of *L. corniculatus* and its pollinators. I want to know: How do pollinator numbers change over time, with respect to the flowering phenology of *L. corniculatus*? How synchronous is the flowering phenology of the species at the population level, and does this affect pollinator behaviour? Can inflorescence architecture also affect pollinator foraging? Does pollinator behaviour apply selection pressures on flowering time? Can the production of large numbers of self-incompatible flowers *en masse* result in reduced fruit-set because of limited pollinator movement and subsequent stigma clogging by self-pollen?

Chapter 5 investigates pre-dispersal seed predation and its effect upon the seed production of *Lotus corniculatus*. I will be asking: What proportion of a plant's seed production is destroyed by pre-dispersal seed predators? Is the proportion constant between years, and are some individuals consistently more heavily predated than others? How does the behaviour of the seed predator species interact with the inflorescence architecture and flowering phenology, both at the individual plant and population levels? Is there any evidence that pre-dispersal seed predation can result in selection pressures to alter flowering time? Can partially eaten seeds germinate, and the seedlings survive?

These are by no means the only ones which could be asked about the reproductive ecology of *Lotus corniculatus*. They do however constitute a logical set of problems, and the interactions between them are ones potentially important in shaping the evolution and ecology of the species. There are six main themes running through this study; *Figure 1.1 is a diagrammatic representation of these* themes, and some of the possible links between them.

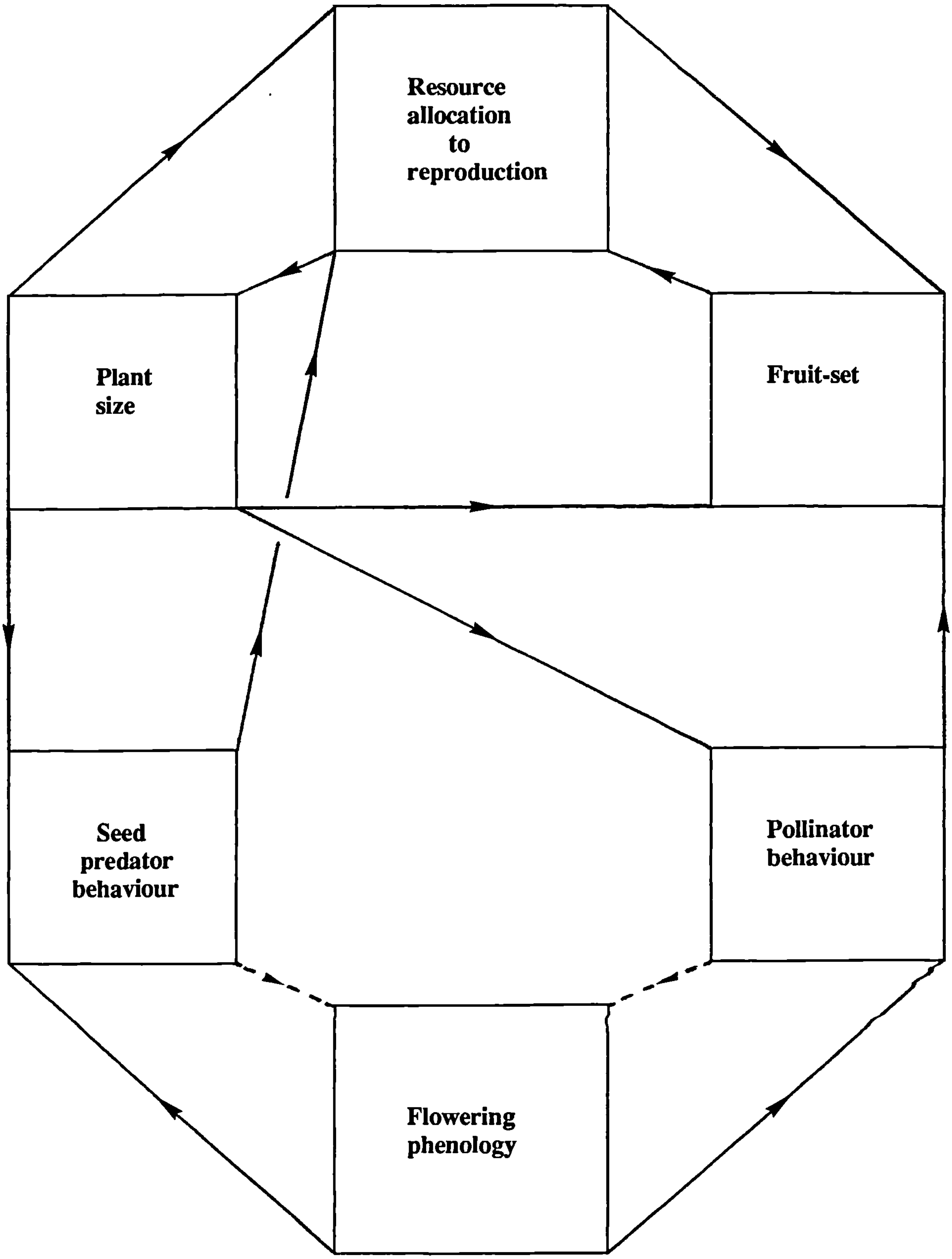


Figure 1.1: The six broad themes of this study, and some of the hypothetical connections/influences between them.

The links shown are the ones pertinent to this study and to the questions already posed. The arrows indicate the direction of the influence (for example, fruit-set may be influenced by pollinator behaviour) and some links are bi-directional (resource allocation to reproduction is likely to affect fruit-set, but fruit-set may influence resource allocation later in the season, or in subsequent seasons). Possible sources of selection pressures are shown by dashed lines (*e.g.* pollinator behaviour may select for plant genotypes with particular flowering times). All of these interactions are hypothetical, and one could prefix "may" before each; there is, though, more evidence for some than for others.

The themes set out in Figure 1.1 cover a wide area of plant reproductive ecology, but there is one overall connecting factor - seed production. The ecological determinants of production within individuals and populations can be intrinsic to the plant, for example patterns of resource allocation, growth habit and size; or extrinsic, such as resource limitation, pollinator behaviour and seed predation. These factors have been looked at by many workers in the field, but we are still far from able to formulate generalisations (if such exist) as to which of them are the most important in affecting individual fitness. As an example, it is known that pre-dispersal seed predators commonly consume a large fraction of the seed crop of individual plants (Sallabanks & Courtney, 1992). Yet Crawley (1992) was able to come to few firm conclusions regarding the effect that this seed predation has on plant population dynamics.

My desire is that a broad-based study such as this will be able to disentangle those aspects of the ecology of a plant species which are important in determining seed output. How successful this has been will be discussed in Chapter 6, where I will give a summation of this work, drawing together the disparate aims into what I hope is an accurate summary of the ecology of flowering and fruiting in *Lotus corniculatus*.

5. A note on statistical analysis

The majority of the statistical work undertaken in this study has been performed using the computer package UNISTAT 4.5 (Unistat Ltd., UK). Three different procedures have been used for testing correlations, depending upon the nature of the data: *ordinary least-squares regression* (when the data have constant residuals); *Pearson's Correlation* (when the residuals are not constant, but the data are normally distributed); *Kendall's Rank Correlation* (when the data are not normally distributed, and residuals are not constant). Inter-year correlations have generally not been attempted between 1990 and 1992 because of the small sample size.

Other tests (*e.g.* χ^2, t-test) have been used as appropriate; the Kolmogorov-Smirnov test for normal distribution has been applied to all of the data. All probabilities quoted are significant at the 5% level, except where indicated.

In some of the phenological graphs, where two or more data series are being compared (for example fruiting phenology and seed predation), one of the data series has been scaled to allow easier graphic comparison. In all cases this has been noted on the y-axis label or in the title.

Chapter 2: Why are Upper Seeds plants so much larger than Lower Seeds Reserve plants?

1. Introduction

2. Methods: Soil analysis

3. Results and discussion: Soil differences

4. Conclusions

1. Introduction

Casual observation of *Lotus corniculatus* at the beginning of this study had indicated that many of the colonising Upper Seeds plants were much larger than the established Lower Seeds individuals. Other species also appeared to follow this trend, for example *Knautia arvensis* (Dipsacaceae) and *Malva moschata* (Malvaceae). Subsequent field work confirmed the observation on *L. corniculatus* (see Chapter 3). The reproductive output of these individuals is a fundamental part of this project and, in an indeterminately flowering species such as *L. corniculatus*, this may be affected by the overall size of the plant; finding out why the Upper Seeds' plants are so large was thus a priority.

There are four possible reasons why the Upper Seeds plants are larger than Lower Seeds Reserve plants:

1. The Upper Seeds plants are older. This is unlikely; individuals of *L. corniculatus* are thought to be "long lived" (Jones & Turkington, 1986) and, as the Upper Seeds field was abandoned in 1981, none of these plants can be more than twelve years old. Lower Seeds Reserve has been an undisturbed grassland for between 25 and 50 years (Gibson, 1986).

2. There are genetic differences between the plants in the two sites. This again is highly unlikely given the length of time that Upper Seeds has been recruiting *L. corniculatus*, and that Lower Seeds Reserve is probably the major source of this recruitment.

3. There are lower levels of inter-specific competition in Upper Seeds because it is a colonising situation. This is certainly possible, but would be very hard to test and is really beyond the scope of this study.

4. The soils of Upper Seeds have a higher nutrient status because of persistent inorganic fertiliser residues, a legacy of the original arable use of the site.

The most likely, and easily testable, of these is hypothesis 4. It is known that prior to its abandonment as an arable field in 1981 Upper Seeds received treatments of inorganic fertiliser. The Lower Seeds Reserve is 25 to 50 years old and may have been cultivated prior to this; the site therefore lends itself as a useful comparison to Upper Seeds. This is the first ever soil analysis of the Upper Seeds site and so it is not possible to say what the present soil nutrient loads are like compared to those twelve years ago at the time of abandonment.

2. Methods: Soil analysis

On one day in April 1992, soil samples were collected from the Upper Seeds and Lower Seeds Reserve sites. The sampling was done on a systematic basis, by pacing out a series of transects running east-west across the two sites. In Upper Seeds samples were taken every 44m along each transect, and the transects were 50m apart; in total, fifty samples were collected, spanning almost all of the field. In Lower Seeds Reserve samples were taken every 10m along each transect, with transects 20m apart; twenty five samples were collected, covering the central portion of the site. These samples were returned to the laboratory where analysis for water content began immediately; subsequent analyses were done over the next two months. The analyses were performed on the basis of the order of decreasing likelihood of changes occurring in the stored soil, and on which analyses could be performed simultaneously. The order was as follows:

1. Water and organic content.
2. pH.
3. Extractable nitrate content.
4. Phosphate content.
5. Potassium and calcium content, and cation exchange capacity.

Standard analytical methods were employed for each analysis; a brief outline is given below, and exact methods can be provided on request.

Water/organic content

The soil samples were weighed, dried at 100°C, then re-weighed to give water content. After heating to 500°C for 24 hours the samples were re-weighed to give an estimation of organic content; a small amount of the mineral fraction is also lost using this technique. The results for water content are expressed as a percentage of the original weight of fresh soil, and those for organic content as a percentage of the weight of dried soil.

pH

The pH of the soil was measured using a glass pH electrode and a pre-calibrated pH meter. The soil sample was first mixed with 20ml distilled water and 5 ml 0.05M $CaCl_2$.

Extractable nitrate

Following a water extraction and subsequent chemical reaction (to bring about a visible colour change), extractable nitrate (*ie* that nitrate immediately available to plants) was measured using a colorimetric method. The results are given as mg extractable NO_3 per 100g dry soil.

Phosphorus

Truog's Extraction was used to determine soil phosphorus availability. This involves an acid extraction, followed by a colour reaction and measurement with a colorimeter. The results are expressed as mg P per 100g dry soil.

Potassium and Calcium

Following extraction with neutral ammonium acetate, potassium and calcium content of the soil was measured using a calibrated flame photometer. The results are expressed as mg per 100g dry soil.

Cation Exchange Capacity

Cation exchange capacity (CEC) describes the "...sum total of exchangeable cations that a soil can adsorb." (Brady, 1990). This capacity is a function of the amount of colloidal material (*i.e.* clay and humus) in the soil. The technique used in this analysis involves saturating the soil's CEC with ammonium ions, then displacing these NH_4^+ ions with barium ions (using barium chloride solution). A titration was performed to find the quantity of NH_4^+ ions displaced into solution. Results are expressed as milliequivalents per 100g dry soil.

All of the chemical analyses were performed on fresh soil, but in order to make them comparable with the other studies, they have been calculated per weight of dry soil using the soil water content results for each sample.

3. Results and discussion: Soil differences

Mean values of the soil analysis results for Upper Seeds and Lower Seeds Reserve are given in Table 1, together with comparable data from three other published studies of calcareous grasslands.

Table 1: Comparison of soil analysis data for Upper Seeds and Lower Seeds Reserve, together with comparable data from three other published studies. The Upper Seeds and Lower Seeds Reserve data have been compared using a separate variance t-test, and the significance valuesare as follows: $^{***}p < 0.001$; $^{**}p < 0.005$; $^{*}p < 0.05$. All means are ±SD.

	Upper Seeds	Lower Seeds Reserve	Limestone dale, north of England[*] north face	Limestone dale, north of England[*] south face	Limestone grassland, North Derbyshire, England[¶]	Vrakelberg[†] (Netherlands)
% water	26.50 ±3.8	30.50 ±2.7***	-	-	-	-
% organic	13.90 ±3.8	17.80 ±2.3***	-	-	28.4	-
pH	6.7 ±0.4	6.7 ±0.2^{ns}	-	-	-	7.9
nitrate	2.36 ±4.5	1.36 ±1.0^{ns}	-	-	4.82[§]	-
phosphorus	2.75 ±3.9	0.65 ±0.3**	0.16	0.20	0.37[§]	1.50 ±0.2
potassium	13.30 ±5.2	10.17 ±2.5**	10.80	12.30	-	13.30 ±1.1
calcium	669.34 ±75.1	617.23 ±66.2**	928.30	749.30	-	399.80 ±12.6
CEC	27.80 ±3.1	31.00 ±2.9***	-	-	-	23.50 ±0.3

sources: [*]Rorison (1990); [¶]Grime & Curtis (1976); [†]Verkaar & Schenkeveld (1984)

[§]determined from samples of fresh soil.

The ranges of most of the Wytham results are broad and the standard deviations high; this is to be expected since localised effects in soils can be great, and it is for this reason that a large number of samples was taken over a wide area in each site.

Water and organic content

Even though the samples from Upper Seeds and Lower Seeds Reserve were collected on the same day, within a period of a few hours, the soil in Lower Seeds Reserve had a higher water content. The larger amounts of organic matter in the soils of Lower Seeds Reserve may be due to the fact that the Reserve has had a larger input of organic material from the persistent vegetation; these higher levels of organic material could account for the greater water holding capacity and CEC. Whether this *c.* 20% difference in organic content is affecting plant growth in Lower Seeds Reserve is not known.

pH

Despite wide fluctuations between samples, the average pH of the soils in the two sites is remarkably similar. The pH of these soils is slightly lower than those of Vrakelberg, perhaps because of the high organic content. Fowler (1993) also looked at the pH of the soils in Upper Seeds and found a mean value (±SD) of 7.6 (±0.17; range = 7.0 - 7.9) compared to my result of 6.7 (±0.4; range = 6.1 to 7.8); soil for this study was also collected in April 1992, so the difference is not due to changes over time. Much more likely is that, because Fowler (1993) sampled over a smaller portion of the site, he did not encounter the extreme fluctuations that I did.

Extractable nitrate

There is no significant difference in the extractable nitrate contents of the soils from the two sites; overall, the levels are low, with a number of samples having no detectable extractable nitrate. Nitrate is soluble and would be unlikely to persist for any length of time following inorganic fertiliser application (Brady, 1990).

This solubility also makes it seasonally variable; the samples were taken after a period of prolonged rainfall which would flush much of the nitrate out of the soil.

Phosphorus

It is the phosphorus levels in the two sites which show the largest differences, with Upper Seeds having more than four times the levels of Lower Seeds Reserve. The only reasonable explanation for this is that it is due to fertiliser residues persisting in the soil. Phosphorus is less soluble and therefore more persistent than nitrate; natural levels of phosphorus are generally low compared with nitrate (Brady, 1990). Comparison with the values for the other published studies shows that Upper Seeds has a far higher phosphorus loading.

Potassium and Calcium

Upper Seeds has significantly increased levels of both potassium and calcium compared to Lower Seeds Reserve. Once again, inorganic fertiliser residues are the most probable cause of the higher potassium levels, though the values found are comparable to those of the Vrakelberg site, and so may not be out of the ordinary for a calcareous grassland.

The increased calcium in Upper Seeds may be a result of the thinner soils on this site, allowing the limestone bedrock to influence this aspect of the soil chemistry. Interestingly, the Wytham values are intermediate between the published values for the English limestone dale and Vrakelberg, showing the wide range of calcium levels found in calcareous grassland soils.

Cation exchange capacity

The higher cation exchange capacity (CEC) in Lower Seeds Reserve may be accounted for by the greater organic content of the soil in that site; humus has a greater capacity to adsorb ions than clay colloids (Brady, 1990). The cation exchange capacity is an important property of soil in terms of plant growth, but a 10% difference in CEC is unlikely to have as profound an effect on plant growth as the 400% difference in soil phosphorus. The CEC for Vrakelberg is comparable to that of the Wytham sites, but nothing is known of the organic content of these Dutch soils.

4. Conclusions

The results of the soil analysis have shown that inorganic phosphorus, and to a lesser extent potassium, are persisting in the soils of Upper Seeds eleven years after its abandonment as an arable field. Phosphorus is an important nutrient for plant growth and plays a "central role...in energetics and protein metabolism" (Ting, 1982). It is frequently in short supply in natural soils (Brady, 1990); for a legume, with its attendant nitrogen-fixing symbionts, phosphate is likely to be much more of a limiting factor than nitrogen. Consistent with these observations is the large size of individuals of *Lotus corniculatus* on the Upper Seeds site. Gough & Marrs (1990) performed a bioassay on the soils of various agricultural and ex-agricultural sites, using *Agrostis capillaris* (Gramineae), *Arrhenatherum elatius* (Gramineae) and *Rumex acetosa* (Polygonaceae). They found that variation in dry weight between plants grown in soils from the sites was best explained by differences in available phosphorus: plants grew larger when there was more available P.

Part of Gough and Marrs' (1990) study was comparable to my own: they contrasted the soil nutrient status of an abandoned field with a semi-natural grassland and found that "...the level of extractable P in the old field soil...has fallen to semi-natural levels in the twelve years or so since the field was abandoned". This is at odds with my finding that levels of phosphorus were appreciably higher in Upper Seeds, compared to Lower Seeds Reserve, eleven years after its abandonment. The reason for this discrepancy may lie with the differences in pH between Gough and Marrs' site (in Sherwood Forest, Nottinghamshire) and the Wytham site. The authors cite rather low pH values of around 3.5 for this area; the parent bedrock is permo-triassic reddish sandstone. Phosphorus availability is much reduced at low pH (Brady, 1990), and most phosphorus is available to plants (and presumably to extraction) within the range pH 6.0 - 7.0, *i.e.* around the mean pH value found in Upper Seeds.

Chapter 3: Reproductive output of *Lotus corniculatus*

1. Introduction

1.1 The investment of maternal resources in plants - an overview

The evolution of life histories in all organisms is concerned with juggling options in an unpredictable world. In plants, this means a compromise between producing the maximum number of offspring, yet providing enough resources for each offspring to have a chance to succeed, and at the same time holding enough resources back to apportion to growth, maintenance and defence of the female parent. Semelparous species, following the onset of reproduction, need only really be concerned with the first two of these compromises; juggling two balls, if you like. Iteroparous species, such as *Lotus corniculatus*, must maintain a three ball juggling act, perhaps over the course of many years. How such life-history strategies evolve in long-lived plants is poorly understood compared to more experimentally accessible ephemeral species (Bazzaz & Ackerly, 1992). Though male function in plants may influence number and quality of offspring (see review in Bertin, 1988), it is ultimately the female function which determines the quantity and quality of seeds produced in any one reproductive episode. This is due to the process of maternal investment which can be defined as the initiation, nurturing and provisioning of offspring by the female parent. In most seed plants this means the number of ovules initiated, how many of the fertilised ovules survive abortion, and the quality of the resulting seeds in terms of resource levels and/or genotype. Resource levels of the seeds may be dependent upon seed size, as a number of studies have shown seed size to be positively correlated with seedling vigour in a wide range of species, including *L. corniculatus* (Carleton & Cooper, 1972); there must be situations, however, where smaller seed size is at an advantage, perhaps because of greater dispersability. Genotype quality can be influenced pre-zygotically or post-zygotically by self-incompatibility systems [see reviews by Barrett (1988) and Seavey & Bawa (1986)] or selective fruit abortion, which again has been documented for *Lotus corniculatus* (Stephenson & Winsor, 1986).

In this chapter three aspects of maternal investment will be examined:

1. How is reproductive output adjusted temporally over the course of one

reproductive episode in terms of the relative importance of inflorescence *versus* individual flower production?

2. How does plant size affect maternal reproductive output?

3. Do the maternal components of reproductive output interact to produce trade-offs between number of fruit per inflorescence, number of seeds per pod and/or seed size?

1.2 Temporal control of flower number

In a seminal paper on the subject of plant maternal investment, Lloyd (1980) set out a hypothesis of control of resources to the maternal component of reproductive output, over the course of one flowering season. He postulated a hierarchical course of potential points of control:

inflorescences ---> flower primordia ---> flowers ---> ovaries ---> fruit

This could be further sub-divided, for example into immature fruit, flower buds, etc. and can be thought of as a continuum [see Primack (1987) for a more substantial discourse on this theme]. At each stage of the process, and at each pertinent point on the plant, there are "...positive or negative responses (investment or no investment)..." (Lloyd, 1980). Factors which will affect the direction of the response include environmental cues, such as drought and predation, and developmental responses, for example how much fruit has already been produced (Stephenson, 1992).

The first level of the hierarchy at which maternal investment is regulated is flower number. Many plants produce more flower primordia than mature flowers, and *Lotus corniculatus* is no exception. From the plant's point of view, flower number is the most manipulable of the possible stages of maternal regulation, and can be varied positively or negatively, in contrast to post-anthesis ovary or fruit numbers, which can only go down. Precisely how a plant adjusts the number of flowers it produces is not clear, but for iteroparous species such as *L. corniculatus* which have a single, or very short, episode of flowering, external cues such as temperature and rainfall, and internal constraints such as resource levels, must be of importance in determining the number of flowers reaching anthesis.

Each level of the hierarchy is a compromise between reproductive output potential and reproductive output realised: for *Lotus corniculatus* there are many more available meristems than produce inflorescences; more flower primordia (typically 7 or 8 according to Hanson, cited in Buzzell & Wilsie, 1964) than flowers per inflorescence (rarely more than 5; *personal observation*); many more ovules than seeds [mean±SD = 42±6.0 (n=31 ovaries); *personal observation*]. Each of these points in the process has its own cues and constraints, but assessing them all, and their interactions with one another, has never been attempted, though some work has been initiated for some species. For example, some authors have documented changes in ovule number per immature fruit over time, which for some species can vary over the course of one reproductive episode, either positively, as in *Diervilla lonicera* (Caprifoliaceae) (Thom son, 1985) or negatively, in *Viscaria vulgaris* (Caryophyllaceae) (Jennersten, *et al.*, 1988).

The aspect of temporal change in maternal investment which will be addressed in this chapter is the relative importance of changes in flower number over time *versus* inflorescence number. In Chapter 1, I outlined Stephenson's (1984) experimental work, in which he found that a cultivar of *L. corniculatus*, under garden conditions, controls investment in flowers at the level of the inflorescence. In this section I wish to test this finding using a natural population. As I stated in Chapter 1, Stephenson and colleagues' work involved cultivars and there are reasons to suspect that the reproductive responses of these plants may be different to their natural ancestors.

1.3 Relationships between plant size and reproductive output

The size-structured nature of most plant populations has been recognised for some time but it is only quite recently that the effects of this situation on the reproductive ecology of a species have been appreciated. Large individuals can have a disproportionate effect on the gene flow and subsequent dynamics of a population through both male and female function because of positive correlations between size and flower production (Dudash, 1991). If there are more resources available to these larger individuals they can be used to increase other components of reproductive output, for example seed size, and so potentially heighten the fitness of these plants and their offspring. Latterly, there has been a focus of attention on seed size with respect to plant size (Venable, 1992), but there are a number of other aspects of reproductive output which could be affected, for example number of seeds per fruit and number of fruit per infructescence. This part of the study will be a survey of these components of female function, and the effect plant size has on them.

Much of the literature concerned with this area of plant reproductive ecology deals in the currency of dry weight, for example the work of de Jong and Klinkhamer, and their colleagues (de Jong & Klinkhamer, 1989; Klinkhamer & de Jong, 1990; Klinkhamer *et al.*, 1992). The debate about which currency to use for such studies has been going on for some time and it was argued by Abrahamson & Caswell (1982) that carbon allocation (*i.e.* dry weight) does not reflect allocation of mineral nutrients to the same tissues. The work of Reekie & Bazzaz (1987a,b,c) showed that, at least for *Agropyron* (now *Elymus*) *repens* (Gramineae), the allocation of nitrogen and phosphorous did correlate with carbon allocation. Whether this conclusion is true of other species is not known. The issue is further complicated by claims that, for some species at least, it may be meristems and not energy and nutrients which are the limiting factor (Watson, 1984). A good, up to date review of this and associated topics is given by Bazzaz & Ackerly (1992). To circumvent this problem, and to provide a non-destructive measure of vegetative biomass (see section 2.4) the currency of reproduction used in this study is whole organs; inflorescences, flowers, fruits and seeds. The currency of vegetative allocation is whole stems and leaves, and their dimensions. Whilst this may not give a fine enough resolution to reproductive effort of the sort required for inter-specific comparisons, it is sufficient for the purposes of comparison between individuals of one species, as in this study.

1.4 Is seed size affected by pod predation?

The possibility exists that seed size may be influenced by predation of other seeds within the same pod; there are two potential hypotheses.

1. **Negative effects:** predation of some seeds in a pod may cause the plant to limit further resource allocation to that pod. If the timing is such that seed development is affected, smaller seeds within predated pods may be the result.

2. **Positive effects:** removing seeds from pods, via predation, could allow a greater share of the resources available to that pod to be distributed amongst fewer seeds, allowing the remaining seeds to grow larger. This would apply also if the limiting resource was space to grow. Once again, timing of predation may be the critical factor.

Is there any evidence for either of these two hypotheses? Ellison & Thompson (1987) in their study of the effects of pre-dispersal seed predators on *Lomatium grayi* (Umbelliferae) found that undamaged seeds from infructescences which had other seeds predated were lighter than those from unattacked ones. The most likely reasons for this, the authors believe, is that the insects either consumed resources destined for the seeds which were not, or could not be, replaced; alternatively, the plant may have shunted nutrients away from the attacked umbels. These two explanations need not be mutually exclusive. Another possibility is that the insects' saliva may have reduced the growth rate of the tissue in the umbel; there is experimental evidence that such a phenomenon occurs between grasshoppers and *Bouteloua gracilis* (Gramineae) (Ellison & Thompson, 1987).

The potential positive effects do not seem to have been considered by anyone, and there is no evidence for or against this in the literature.

1.5 Trade-offs between aspects of reproductive output

All models of resource allocation in plants assume that in any one year there will be a limited quantity of the resources necessary to an individual, available for maintenance, growth and reproduction. At the heart of resource allocation theory is the idea that a plant's resources are finite and that a compromise between the quantities of these resources which are allocated to different uses must be reached. The exact regime of this compromise (or "trade-off") will be determined by a variety of factors. Possible resources may be: water, energy (photosynthate), mineral nutrients, micronutrients, or meristems (Lovett Doust, 1989).

Resource trade-offs have only occasionally been looked at within individuals; most work has been done between individuals of the same species, either within or between populations. Reasons for this lie with the historical wholesale transfer of resource allocation theory from animal studies to plant research. Animal studies have shown that different offspring number:size ratios may represent adaptive "strategies" (Stearns, 1977), and such inter-specific evolutionary insights are considered more informative than ecological case studies. This approach has not been so successful in plants. One reason is because within-population plant sizes can vary by several orders of magnitude, obscuring patterns of resource allocation in those species in which individual size is correlated with maternal components of reproductive output, for example fruits per infructescence, seed number per fruit or seed size (Samson & Werk, 1986). This study will examine resource allocation and trade-offs within individuals of *L. corniculatus*.

Infructescence organisation can be viewed as having the following components:

1. Number of fruit per infructescence.
2. Number of seeds per fruit.
3. Mean size of seeds per fruit.

The hierarchical nature of this relationship is not simply physical; there is also a hierarchy of possible trade-offs. The simplest model would predict that an increase in numbers at one level would result in a negative correlation with the next lower level, and perhaps subsequent levels. For example, an increase in numbers of fruits per infructescence would lead to fewer seeds per fruit (Figure 3.1).

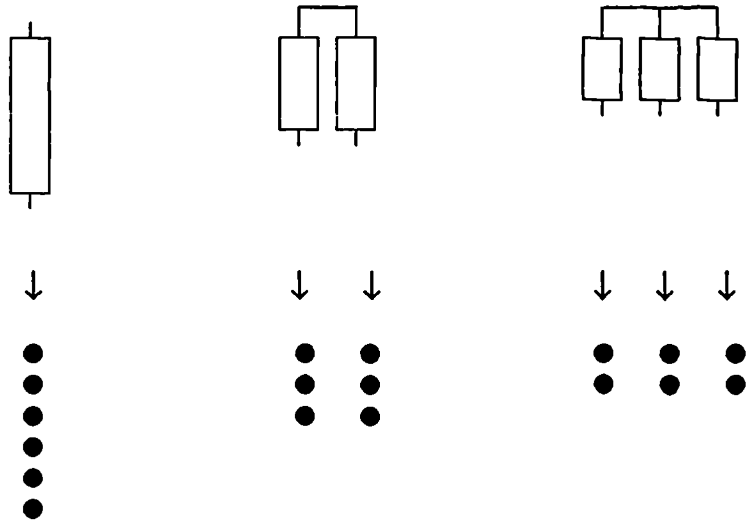


Figure 3.1: Hypothetical trade-off between number of fruit per infructescence and number of seeds per fruit.

Another possibility is that more seeds per fruit would correlate with reduced seed size (Figure 3.2).

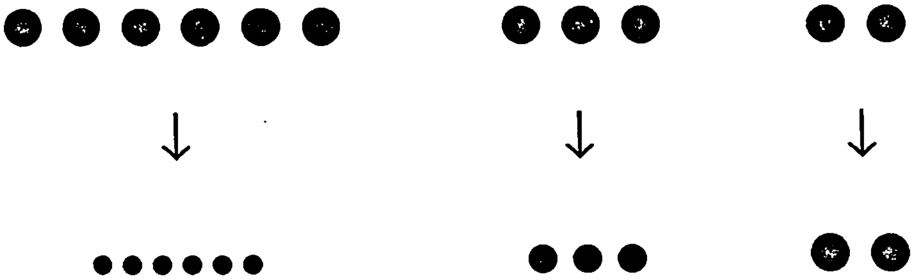


Figure 3.2: Hypothetical trade-off between number of seeds per fruit and seed size.

Or, these two factors may interact to produce a situation where more fruit per infructescence results in fewer, smaller seeds (Figure 3.3).

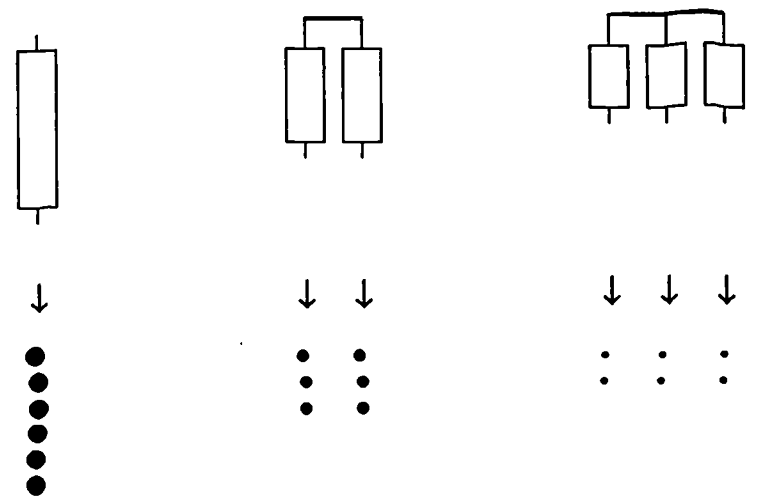


Figure 3.3: Hypothetical interaction between fruit per infructescence, seed number and seed size.

Another view point to resource allocation is provided by physiological studies of reproductive allocation in plants. There is much evidence to suggest that, for photosynthetic resources at least, there is some degree of structural autonomy within an individual. For example, in a study of *Gymnocladus dioicus* (Leguminosae) Janzen (1976a) showed that defoliating particular branches on a plant causes the abortion of fruit initiated on those branches. Similarly, Waller (1982) found that those seeds produced at the top of plants of *Impatiens capensis* (Balsaminaceae) were heavier than those further down; this can be explained by seeds at the top benefiting from increased availability of photosynthate. It is clear from these and other studies that localised allocation of photosynthate in flowering plants is the norm (Watson & Casper, 1984), and it is more useful to regard most plants as collections of "Integrated Physiological Units" (Watson, 1986).

The situation with regard to mineral nutrients is less clear, but one can imagine that nutrient resources entering the plant via the roots are available to the plant as a whole, and must be partitioned amongst the various resource "sinks". How this is achieved is not clear, though most workers visualise it as internal competition, with the stronger sinks gaining more resources, such that those infructescences with the greatest number of developing fruits act as stronger sinks.

Within any inflorescence or infructescence, resources must be partitioned between individual flowers or fruits; the process continues for individual fruits, as there must then be a division of nutrients between the seeds. If there really is competition for resources, then the stronger sinks will take more; the question is - is "more" proportional to the number of fruit, or out of proportion? One way of answering this may be to look at variation in seed mass within a plant. If larger infructescences are drawing to themselves disproportionate amounts of resources, then we might expect the mean seed weight of these infructescences to be heavier. However, if they are drawing resources in proportion to their number of fruit, we may expect it to be the same. If the resources are not dependent upon numbers of fruit, and so the same amount of resources needs to be divided between more fruits, seed weight could be reduced.

The results of the few previous studies are confusing and contradictory. Mazer *et al.* (1986) reported negative trade-offs between seed size and number of seeds per fruit in *Raphanus raphanistrum* (Cruciferae), though results were combined from a number of individuals, and the study predated the warnings of Samson & Werk (1988) on the dangers of merging data from different sized individuals. Michaels *et al.* (1988) reported a negative trade-off for 7 of 10 individuals of *Asarum canadense* (Aristolochiaceae), and a positive correlation for 5 of 6 individuals of *Staphylea trifolia* (Staphyleaceae); individuals of the other 19 species looked at showed no significant trends. The fact that only some of the individuals of the two species did show significant correlations is suggestive. It could be that the nature of the patterns of resource allocation is dependent upon the resource status of the individual plant. I am hypothesising that individual plants with a surplus of resources may show positive correlations between the factors outlined above; those plants with just enough resources would show negative correlations; plants with a deficit of resources may show no correlations between the components of reproduction and simply reduce their overall investment in the number or size of those components. Plant size is the measure of resource status which will be used.

2. Methods

2.1 The individual plants being studied

The majority of the research described here has been undertaken using individual, marked plants of *Lotus corniculatus*. Plants were chosen according to the following criteria.

1. **Size:** a range of plant sizes was selected to enable the relationship between size and reproductive output to be established.

2. **Position:** plants were chosen which as far as possible reflected the geographic range of this species in the four sites.

3. **Discreteness:** the problem of "what is an individual" is a perennial one in plant ecology. Plants were selected on the basis of their distance from other individuals and, particularly in the case of the larger plants, on their shape; *L. corniculatus* grows from a central perennating point and there is little rooting of branches, particularly if the plant is not being grazed (*personal observation*). Consequently, single individuals tend to be more or less circular in shape. These were the plants chosen.

The individual plants were given permanent identification numbers, with letter prefixes indicating which site they were from *eg.* **LU**...or **LP**...Upper Seeds; **LR**..Lower Seeds Reserve; **LC**...Rough Common; **LQ**...The Quarry. In this and subsequent chapters identification numbers with these prefixes will occasionally be used on some of the phenological graphs for comparison of the same individuals between years.

Small numbers of plants were used in 1990; though every effort was made to utilise the same plants from year to year, the inevitable vagaries of death and deer activity conspired to reduce the between-years sample sizes. Consequently it has not been possible to perform some between-years analyses of the data; these have been marked with a dash (-) in the tables.

2.2 Flower censusing

The number of flowers produced by each of the plants in each of the three years was counted at regular intervals, with most censuses taking place every 7 days or so. In 1990, all of the flowers were marked with ink as they were counted; from this, it was found that the flowers usually last about 7 days, depending upon weather conditions and perhaps whether they had been pollinated, and so the condition of a flower is a good guide to its age. Marking was not performed in 1991 and 1992; any "old" flowers were considered to have been counted in the previous census. Though subjective, I do not believe this method to be inaccurate as, after one full season of marking (1990), I felt confident in my ability to estimate the age of flower, and the probability of its having already been counted. All flowers were counted, except in 1991 and 1992 when quadrat samples were taken on large plants during times of high flower production. The data were recorded as numbers of flowers in each inflorescence, giving a count of total flower and total inflorescence numbers. Often, an inflorescence was encountered in which some of the flowers were open and others closed; in this event, the inflorescence was included in that day's census. The precise period between flower censuses was not vital, as the important measure from the point of view of this study was the rate of flower production *i.e.* the number of new flowers produced per day. This was calculated from the total number of new flowers censused on a particular day divided by the number of days since the last assessment. All flowering profiles subsequently plotted use these data.

2.3 Fruit collecting

In 1990 all of the fruits (pods) of the plants being studied were collected. In 1991, except for the smallest plants, all of the fruits from a permanent quadrat within each plant were collected; the size of the quadrat varied with the size of the plant. Fruits from tagged inflorescences (see **Methods** Chapter 4) were also collected. In 1992 only the fruits produced by the tagged inflorescences were gathered. In each year the infructescences were individually bagged for later processing in the laboratory. Fruit were dissected as soon as possible after collecting, and split pods were not used for determining number of seeds per pod.

For each infructescence the following information was collected (exceptions are noted in parentheses):

1. Number of pods per infructescence.
2. Length of each mature, intact, unshrivelled pod (except 1990).
3. Number of undamaged seeds per pod.
4. The combined weight of the undamaged seeds in each pod (in 1990 seeds were weighed individually); seeds were handled with fine forceps.
5. The identity and numbers of any seed predators in each pod.

2.4 Measuring the size of individual plants

The usual way of assessing the size of a plant is by measuring biomass; the plant is harvested, dried to constant weight, and this gives a measure of how large the individual was in terms of total fixed carbon (but does not say anything about differences in, for example, leaf size or stem length between individuals). For the purposes of this study, in which the same plants would be monitored over a period of two or three years, a destructive method of deducing plant size was not feasible. Consequently, a non-destructive measure had to be developed. This involved producing a Biomass Index for each plant comprising the following elements:

1. **Total number of stems per plant** was counted either by direct counting, if the plant was small enough, or by sampling using a 10cm x 10cm quadrat. For the latter the number of samples taken was dependent upon the size of the plant; larger plants had more quadrats taken. To calculate the total number of stems possessed by the individual, it was necessary to estimate the area of the clone. This was done by measuring the circumference of the plant, which in most cases was roughly circular. Using simple circular geometry the area of the clone can be estimated and an approximation of the total number of stems calculated.

2. **Mean stem length** was calculated by measuring a sample of stems in the field. The size of the sample was not fixed, but was determined according to the variability of stem lengths; measurements were taken until the calculated mean ceased to be greatly affected by the addition of new lengths to the total.

3. **Mean number of leaves per stem** was calculated by counting the number of leaves present on the stems measured above.

4. **Mean leaf length** also involved measuring a sample; sample size was again determined by the variability of the measurements. There is a strong positive correlation between leaf length and width ($r^2=0.92, p<0.001$), therefore measuring one axis is sufficient.

All of these elements can be combined to give:

$$\text{Biomass Index} = \begin{array}{l}\text{Total No.}\\ \text{Stems}\end{array} \times \begin{array}{l}\text{Mean Stem}\\ \text{Length}\end{array} \times \begin{array}{l}\text{Mean No.}\\ \text{Leaves}\\ \text{per Stem}\end{array} \times \begin{array}{l}\text{Mean Leaf}\\ \text{Length}\end{array}$$

A biomass assessment was made for all plants in 1990 and, because of problems with deer grazing, most plants in 1991.

In order to test the accuracy of this measure of biomass, in June 1993 5 Upper Seeds plants and 5 Lower Seeds Reserve plants had their Biomass Indices calculated and their above ground biomass harvested. The reproductive tissue was removed from the samples and the remaining stems and leaves dried to constant weight at 100°C. Biomass Index for each plant was then plotted against actual above ground biomass (Figure 3.4); the relationship is a linear one and the two components are highly correlated ($r^2=0.997, p<0.001$). I would be wary of using these data to calibrate the biomass index such that the actual biomass could be calculated from the index: it would be no surprise if the relationship (*i.e.* the slope of the line) changed from year to year.

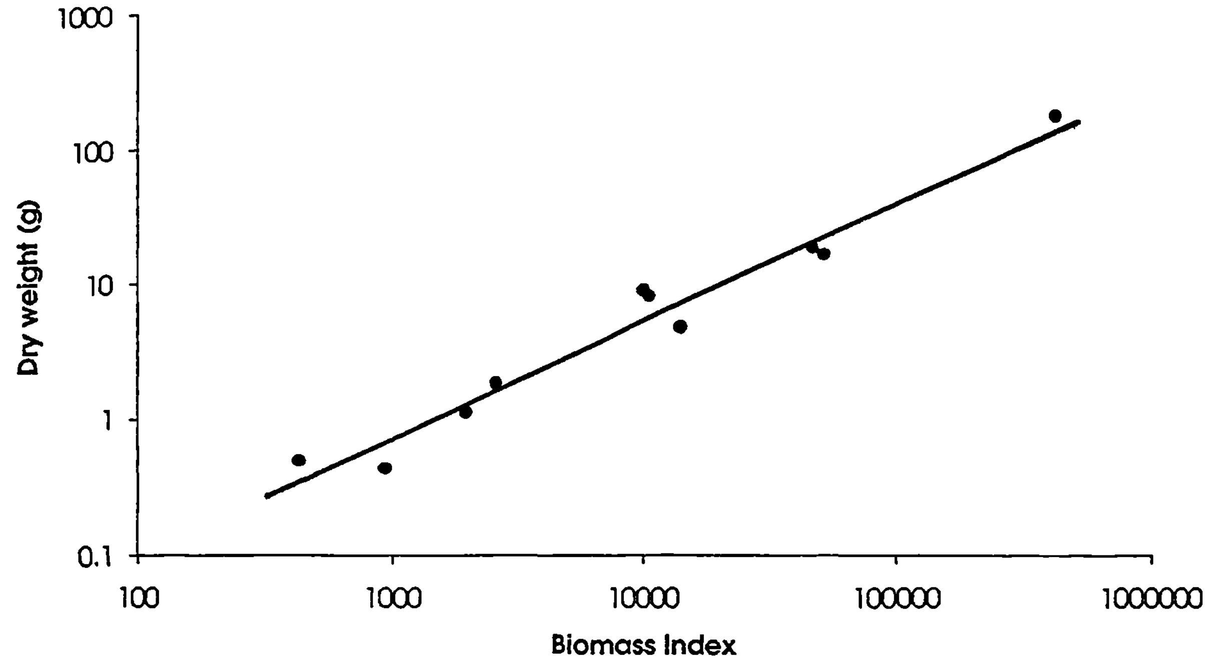


Figure 3.4: The relationship between above ground biomass and biomass index for 5 Upper Seeds and 5 Lower Seeds plants, 1993.

3. Results

Due to the paucity of some of the data from the established sites, certain analyses could only be carried out on Upper Seeds' plants; where this is the case, it has been noted in the title to the table or figure. All phenological data in this and subsequent chapters use day of the year as the x-axis, starting with 1st January as 1, and 31st December as 365 (or 366 in a leap year).

3.1 Biomass assessments

Biomass assessments of the Upper Seeds' plants ranged from 599 to 43686 in 1990; and from 1878 to 1514961 in 1991. Biomass indices for Lower Seeds Reserve plants ranged from 146 to 3487 in 1990; and from 142 to 6680 in 1991. Plants in The Quarry and Rough Common were assessed in 1991 only; the range for The Quarry was 44 to 3240, whilst that for Rough Common was 106 to 1678. These results confirm the observation that plants in the colonising site tend to be much larger than those from established sites in Wytham.

3.2 Temporal regulation of flower number

Flower production was generally so low in the Lower Seeds Reserve plants that plotting changes in mean number of flowers per inflorescence is meaningless, because the data can be biased by a few large inflorescences. Therefore, all of the following analyses use Upper Seeds plants only. A small sample of the Upper Seeds plants is shown in Figure 3.5. Mean flowers per inflorescence does vary over time; the range of means in these plants is 1.3 - 3.9 flowers per inflorescence. Some of the extremes of this range are due to there being few inflorescences produced at those times, resulting in spuriously large or small means; this does not account for all of the variation, however - see, for example, plant LU13 in 1992. Pearson Correlations between mean flowers per inflorescence and rate of inflorescence production were performed on the combined data from Upper Seeds plants in each of the three years, but excluding those dates with rates of inflorescence production smaller than 10 inflorescences per day, to avoid biases caused by a small sample size. None of these correlations was significant, indicating that inflorescence size does not vary predictably with rate of inflorescence production.

Averaging the data for mean flowers per inflorescence over all plants (again omitting censuses with fewer than 10 inflorescences) allows us to look at site trends (Figure 3.6). In 1990 the rather small sample size means that confidence intervals are large and there are too few data at the very end of the season (when inflorescence production is low and most plants are producing fewer than 10 per census) to make analysis at this period viable. Nevertheless, mean number of flowers per inflorescence does not vary much over the flowering period. In 1991 and 1992, on the other hand, mean flower number declines significantly over the course of the season.

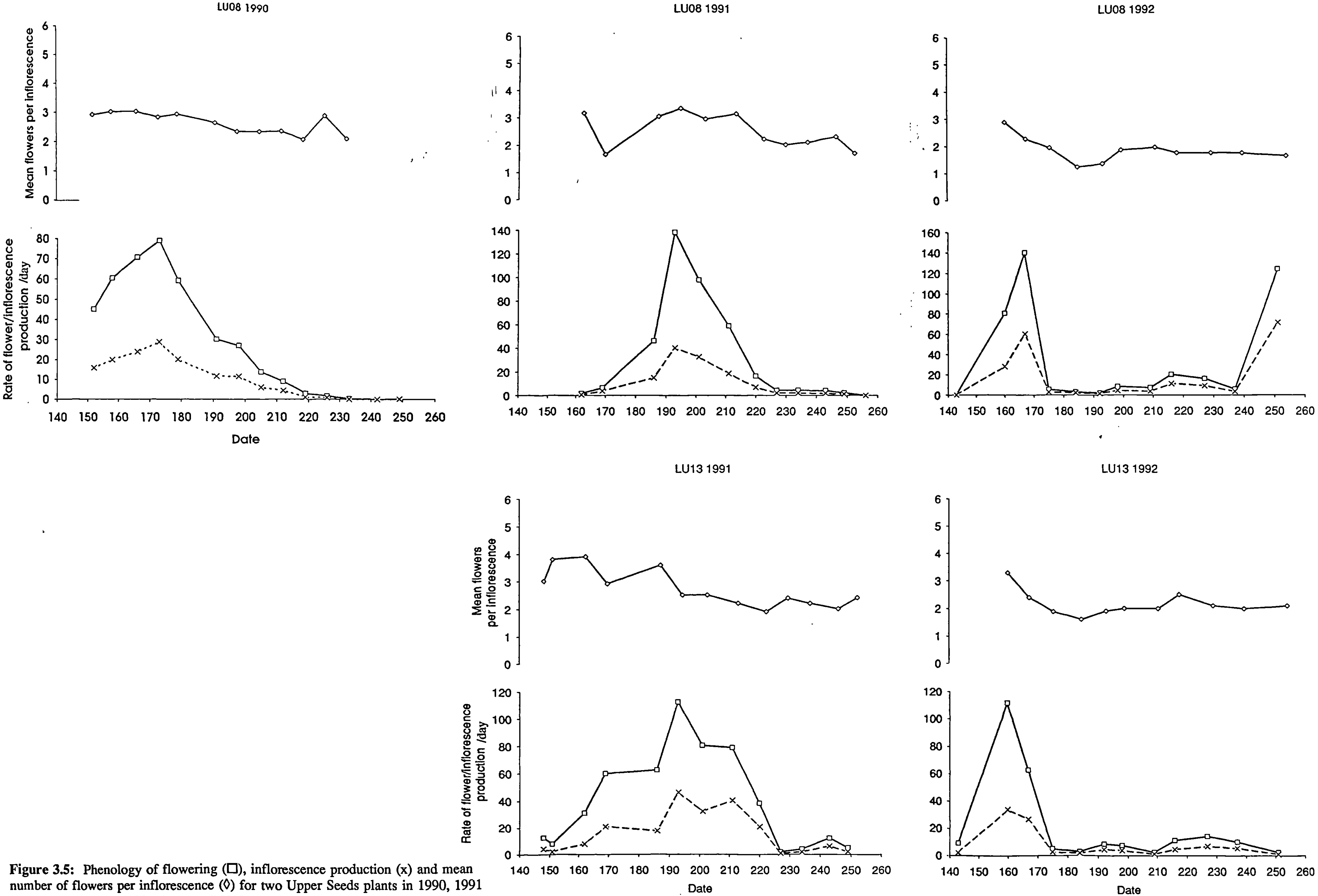


Figure 3.5: Phenology of flowering (□), inflorescence production (x) and mean number of flowers per inflorescence (◊) for two Upper Seeds plants in 1990, 1991 & 1992 (LU08) and 1991 & 1992 (LU13).

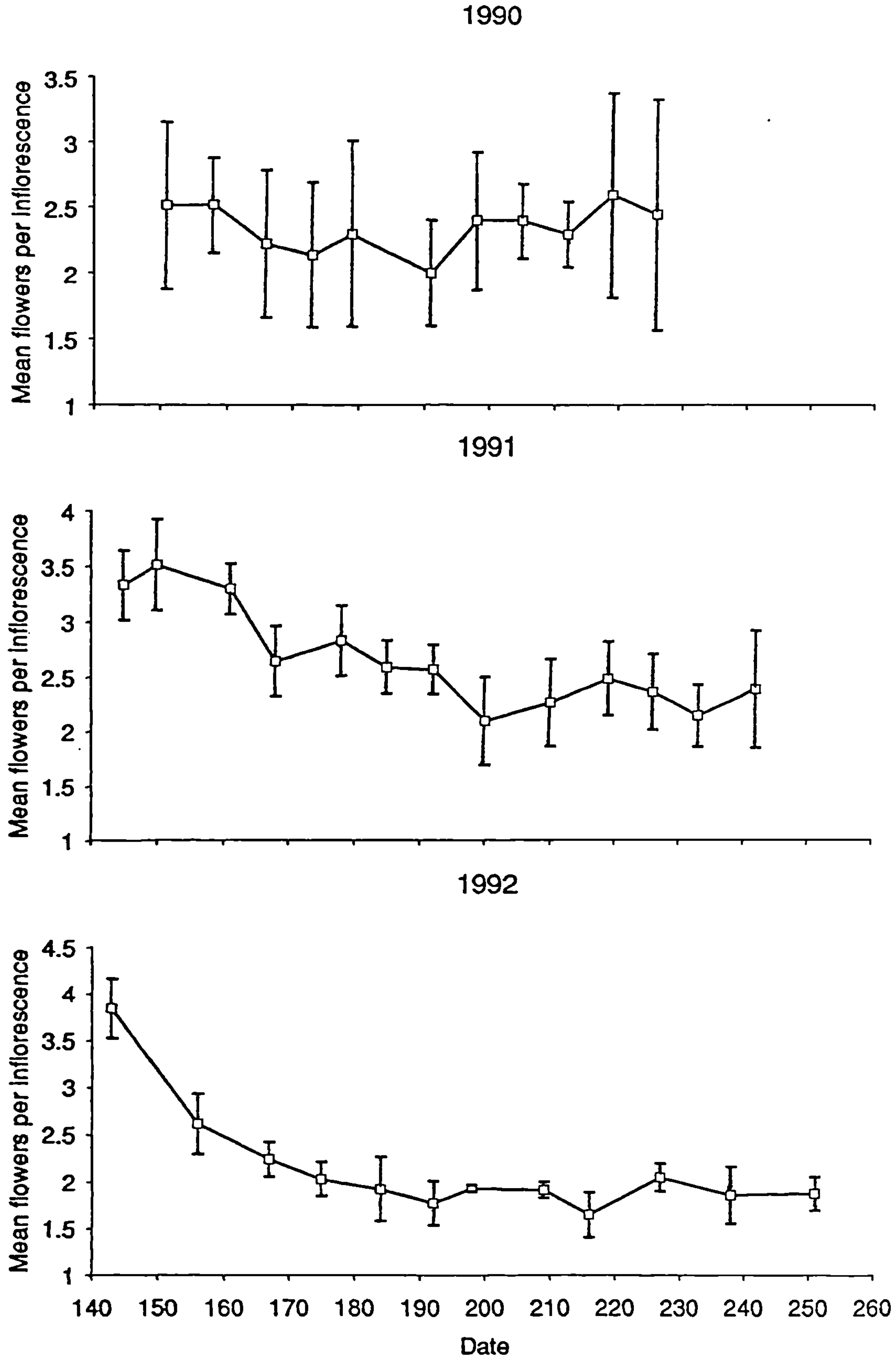


Figure 3.6: Changes in mean number of flowers per inflorescence over time in Upper Seeds in the three years of the study. Error bars signify 95% confidence limits.

The frequencies of the six different classes of inflorescence size, averaged for all individuals in Upper Seeds in each year, are shown in Table 3.1. In each of the three years the majority of the inflorescences are of the smaller size classes.

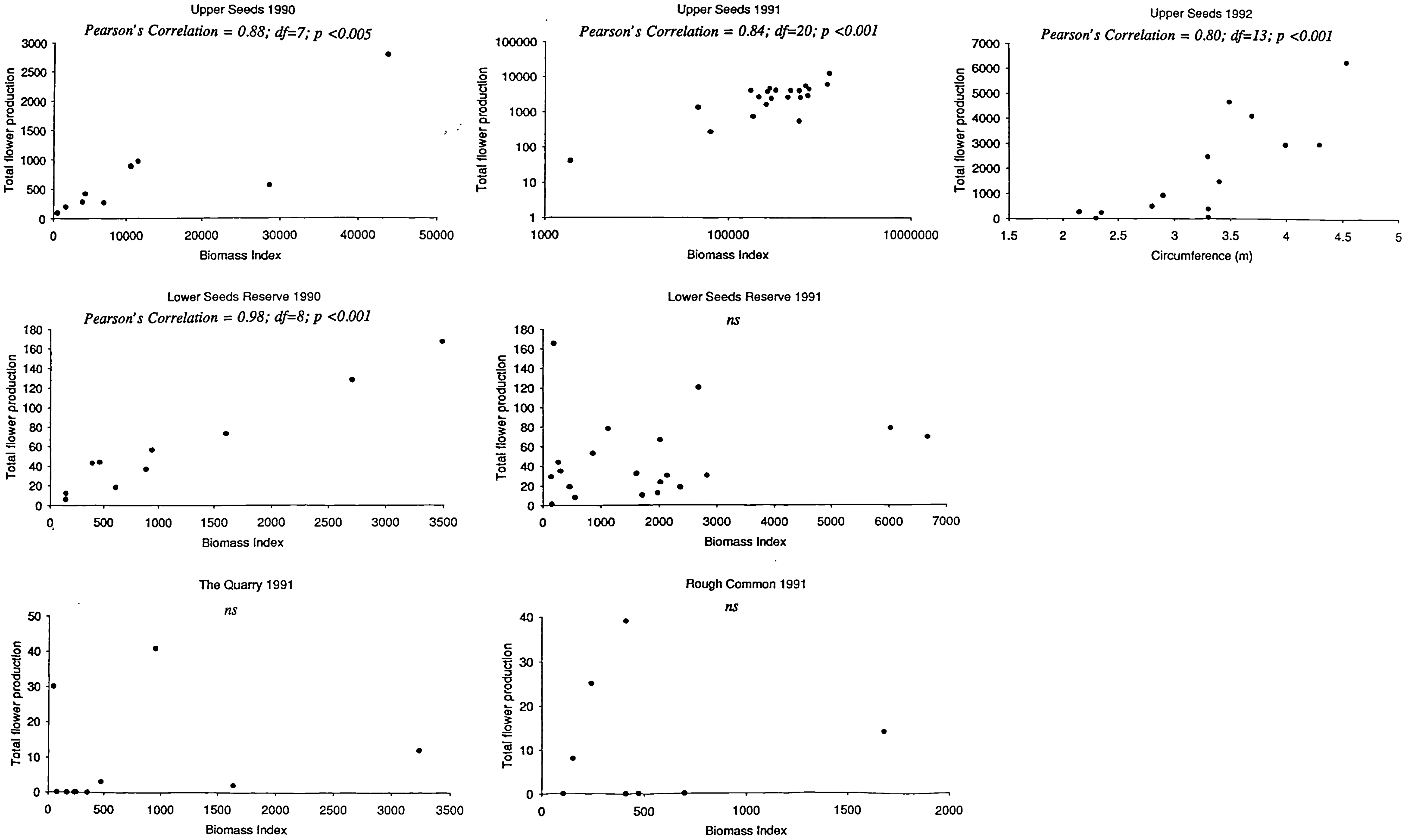
Table 3.1: Average frequency of different size classes of inflorescences for Upper Seeds plants in the three years of the study. All figures are mean % (±95% confidence limits).

	Number of flowers per inflorescence:					
	1	**2**	**3**	**4**	**5**	**6**
1990	31.4 (±3.9)	25.3 (±1.2)	24.1 (±1.0)	14.4 (±1.9)	4.7 (±1.2)	0.2 (±0.2)
1991	23.0 (±3.5)	24.3 (±1.5)	20.6 (±0.8)	17.7 (±1.0)	11.4 (±1.5)	3.0 (±1.3)
1992	39.1 (±3.1)	29.1 (±1.2)	19.0 (±1.5)	9.4 (±1.7)	3.2 (±0.8)	0.5 (±0.2)

3.3 The effect of plant size on reproductive output

The relationship between biomass index and total flower production of each individual plant in the four sites in 1990 and 1991 is shown in Figure 3.7, together with statistical correlations. In 1992 no biomass assessment was made of the plants; however, there was a correlation between total flower production and plant circumference in Upper Seeds. The slopes of the lines of the significant relationships were compared to one another using a modified t-test (Zar, 1984), both within years and between years. None of these comparisons was significantly different from one another.

Between-years, flower production can vary for the same individuals (Figure 3.8). Flower production in 1992 appears to be less relative to the previous two years.



Upper Seeds 1990

Pearson's Correlation = 0.88; df=7; p <0.005

Upper Seeds 1991

Pearson's Correlation = 0.84; df=20; p <0.001

Upper Seeds 1992

Pearson's Correlation = 0.80; df=13; p <0.001

Lower Seeds Reserve 1990

Pearson's Correlation = 0.98; df=8; p <0.001

Lower Seeds Reserve 1991

ns

The Quarry 1991

ns

Rough Common 1991

ns

Figure 3.7: The relationship between plant size and flower production, in all sites in 1990 and 1991. The significance of the correlations is as indicated.

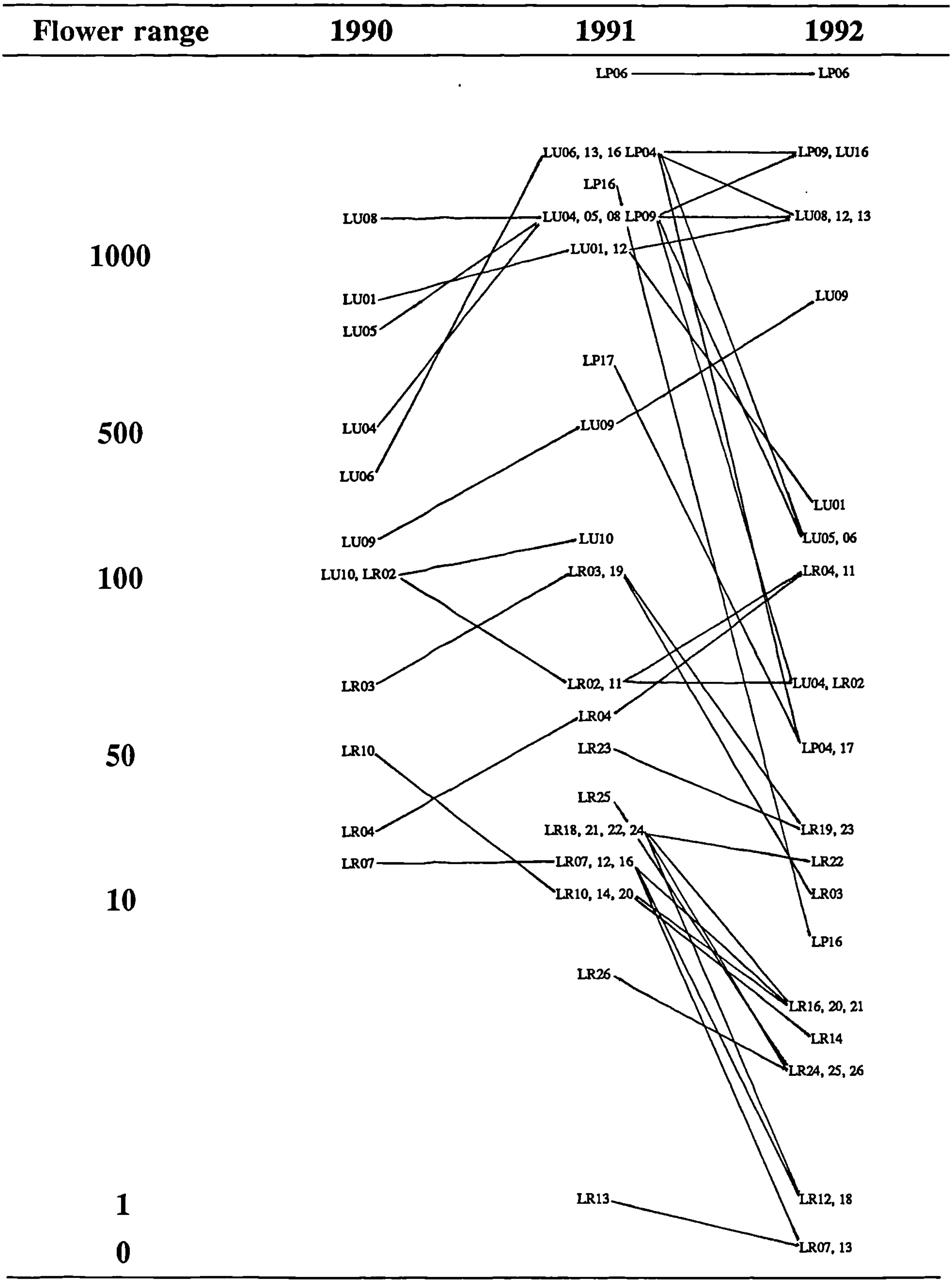


Figure 3.8: Changes in flower production for the same Upper Seeds and Lower Seeds Reserve plants in 1990, 1991 and 1992

Correlations between plant size and total pod production have been performed (Table 3.2). Only in Lower Seeds Reserve in 1990 is this relationship not significant.

Table 3.2: Correlations between plant size and pod production, all sites in all years.

Site	Year	Pearson's Correlation	df	significance
Upper Seeds	**1990**	0.86	7	p <0.002
Lower Seeds	**1990**	-	-	ns
Upper Seeds	**1991**	0.50†	-	p <0.002
Lower Seeds	**1991**	0.68	18	p <0.001
Rough Common	**1991**	0.61	6	p =0.05
The Quarry	**1991**	0.79	8	p <0.005
Upper Seeds•	**1992**	0.51	13	p <0.03

†Kendall Rank Correlation.
•Calculated using flower production as a measure of plant size.

The proportional reproductive output of Upper Seeds and Lower Seeds Reserve plants has been calculated in two ways: as total flowers/biomass and as pod production/biomass. The relationship of these measures of proportional reproductive output to plant size is shown in Table 3.3. Using flower production, negative correlations are obtained in Upper Seeds (1990 and 1991) and Lower Seeds Reserve (1991); using pod production, proportional reproductive output does not correlate with biomass on any occasion.

Table 3.3: Proportional reproductive output, assessed by flower and fruit production, for Upper Seeds and Lower Seeds Reserve in 1990 & 1991.

Site	Year	Flowers/Biomass v Biomass	Pods/Biomass v Biomass
Upper Seeds	**1990**	Pearson's Correlation = -0.54; df=7; p=0.067	ns
Lower Seeds	**1990**	ns	ns
Upper Seeds	**1991**	Pearson's Correlation = -0.41; df=20; p <0.05	ns
Lower Seeds	**1991**	Kendall's tau= -0.42, p <0.005	ns

The relationship between the proportion of flowers setting fruit and total flower production for individual plants in each of the three years is shown in Figure 3.9 (Upper Seeds) and Figure 3.10 (Lower Seeds Reserve). In both sites, in all three years, the only linear correlation between the two components is in Upper Seeds, 1992 (*Pearson's Correlation = -0.50; df=13; p <0.03*).

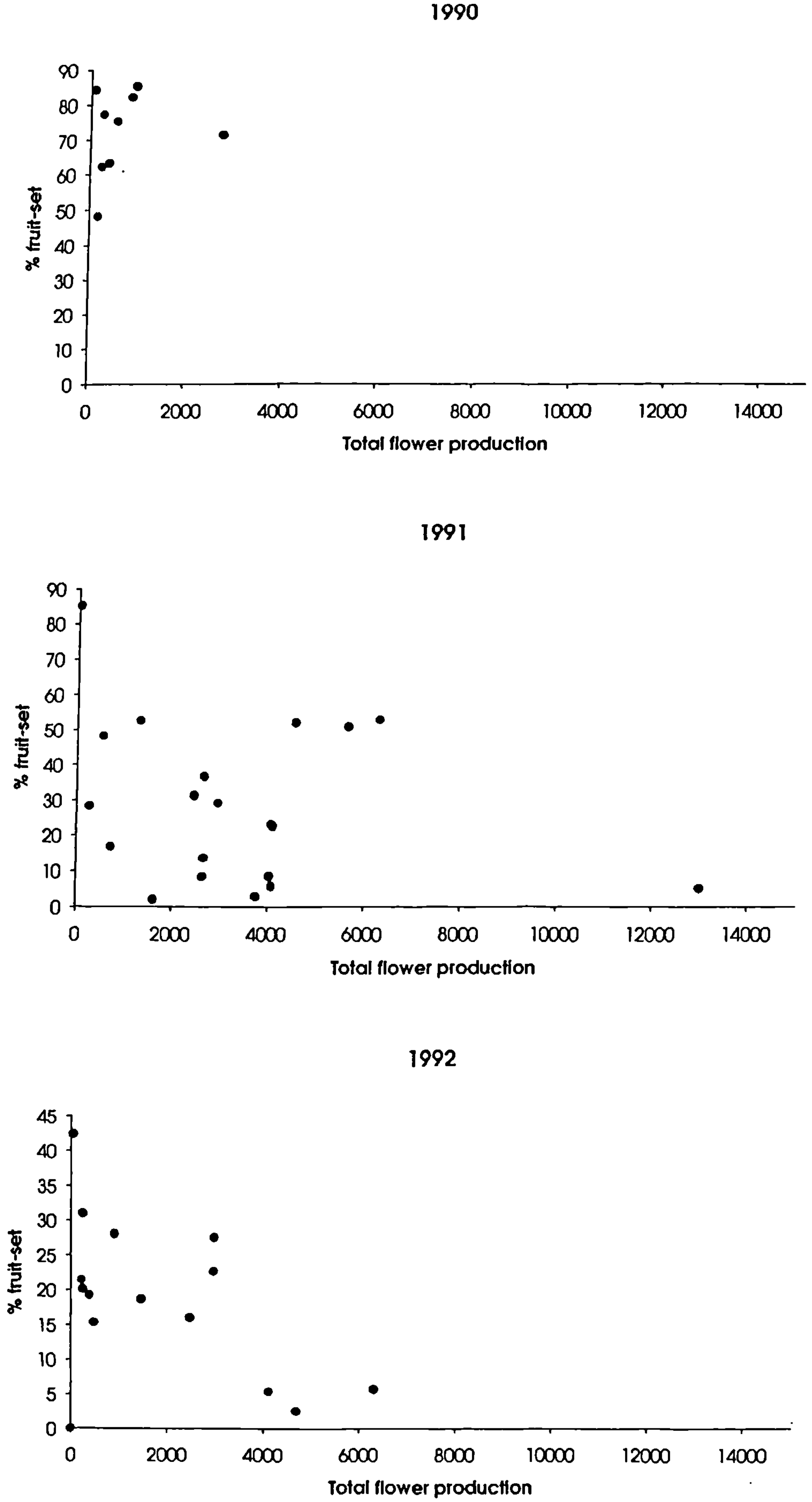


Figure 3.9: The relationship between flower production and proportion of fruit-set, Upper Seeds in 1990, 1991 & 1992.

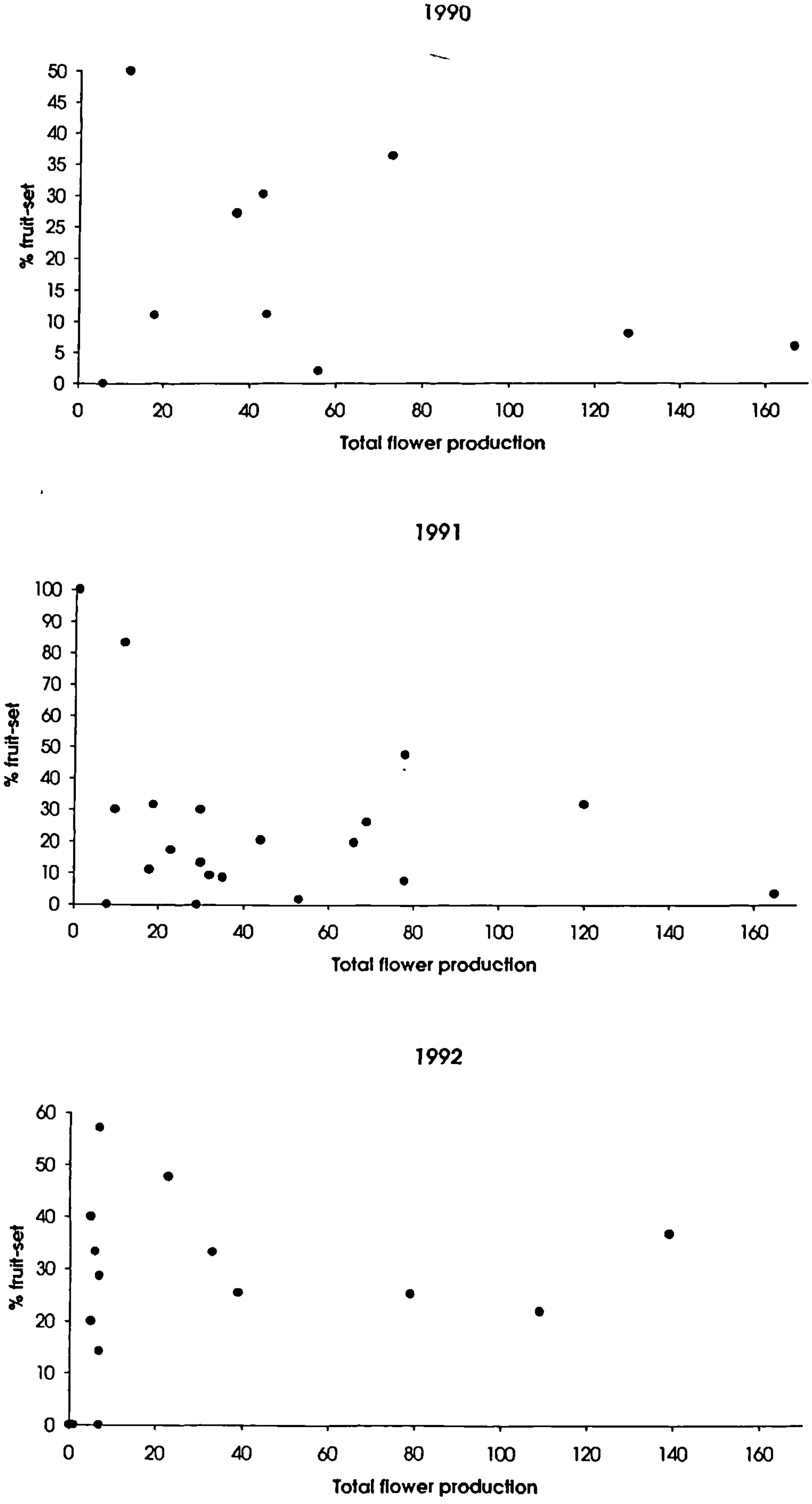


Figure 3.10: The relationship between flower production and proportion of fruit-set, Lower Seeds Reserve in 1990, 1991 & 1992.

Inter-year variation in percentage fruit-set is given in Figure 3.11 (for Upper Seeds plants) and Figure 3.12 (for Lower Seeds Reserve plants). A reduction in fruit-set between 1990 and 1991 is apparent for the Upper Seeds plants; there are no trends for the Lower Seeds plants.

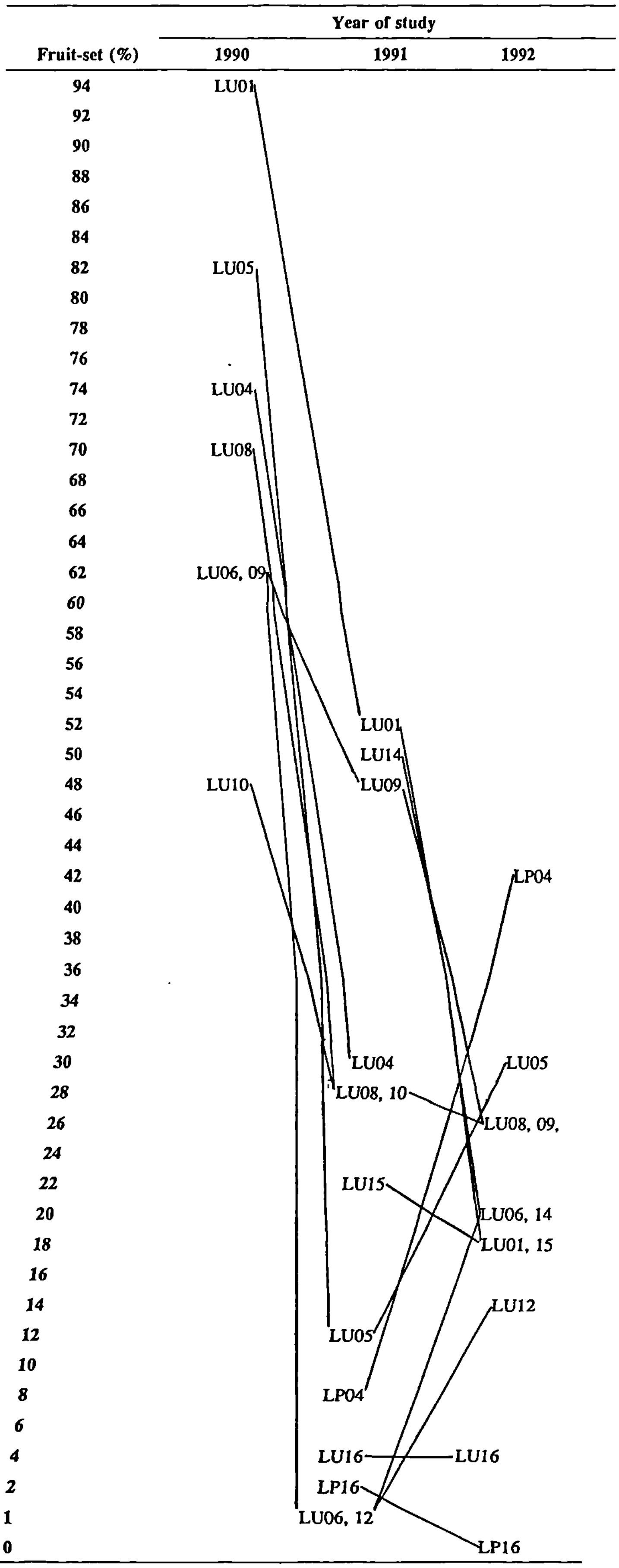


Figure 3.11: Variation in percentage fruit-set for Upper Seeds individuals in 1990, 1991 and 1992.

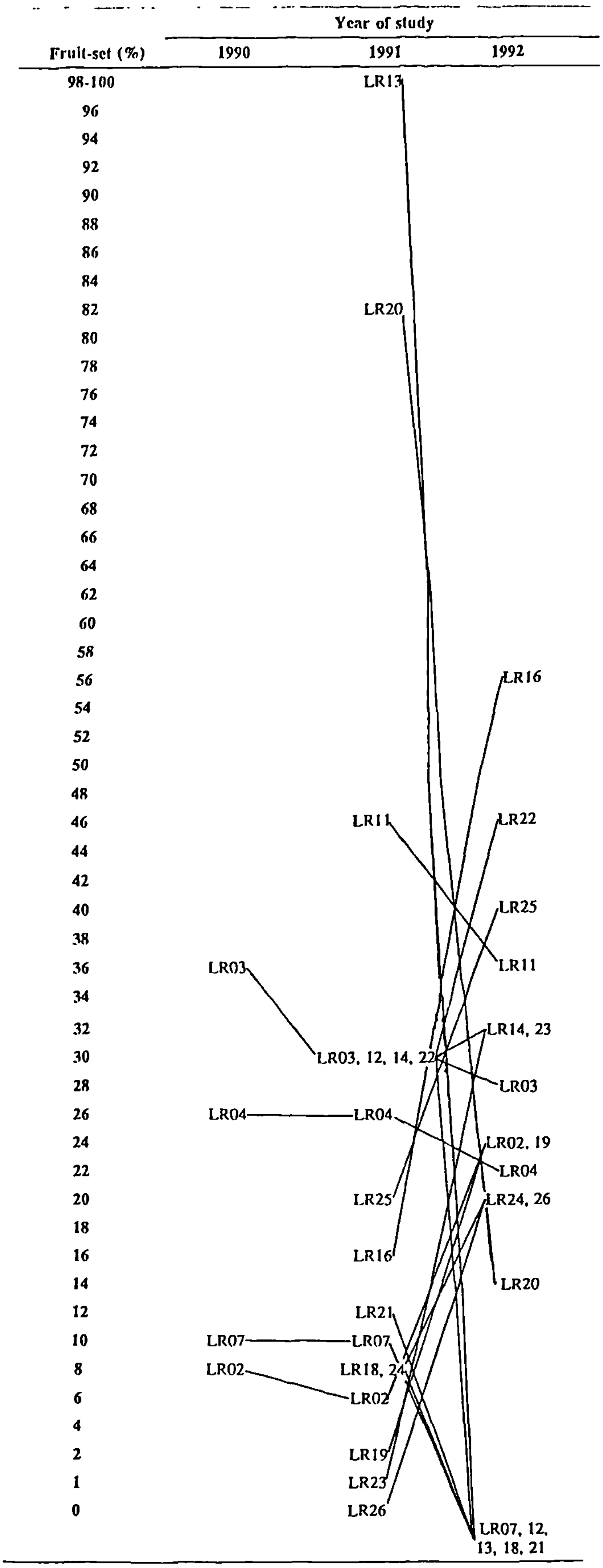


Figure 3.12: Variation in percentage fruit-set for Lower Seeds Reserve individuals in 1990, 1991 and 1992.

Seed size and seed predation

The mean seed weights of seeds from predated and unpredated pods were compared for a number of plants in Upper Seeds (Table 3.4); seed samples from plants in Lower Seeds Reserve and the other established sites were too small to allow this analysis.

Table 3.4: Comparison of mean seed weights from predated and unpredated pods for 5 plants in Upper Seeds; sample sizes are given in parentheses. The data have been tested using separate variance t-tests All data are from 1991 except where indicated; significance is at the 5% level. Sample size refers to number of seeds weighed.

	Mean seed weight (g)		
Plant	**predated pods**	**unpredated pods**	**significance**
LU01	0.0011 (n=48)	0.0013 (n=94)	ns
LU01 (1992)	0.0012 (n=40)	0.0012 (n=50)	ns
LU04	0.0013 (n=168)	0.0013 (n=50)	ns
LU14	0.0015 (n=123)	0.0015 (n=77)	ns
LP15	0.0017 (n=75)	0.0017 (n=65)	ns

The relationship between plant size and other components of reproductive output

Plant size was correlated with mean flowers per inflorescence only in 1992[1] (*Pearson's Correlation* $= 0.84$; $df=13$; $p < 0.001$). Plant size was not correlated with mean pods per infructescence in 1990, 1991 or 1992. Mean number of seeds

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[1] All 1992 correlations have been calculated using total flower production as a measure of plant size.

per pod[2] was not related to plant size in 1991 or 1992 (1990 omitted due to small sample). Seed weight was positively correlated with plant size in 1990 (*Pearson's correlation = 0.76; df=7; p <0.01*), and almost significantly correlated in 1991 (*Pearson's Correlation = 0.44; df=9; p=0.089*) (1992 omitted because of small sample).

Between-years correlations of the other components of reproductive output

For the same plants in Upper Seeds and Lower Seeds Reserve, between-years correlations have been performed using the data for mean number of flowers per inflorescence, mean pod length, mean number of pods per infructescence and seed weight. The only significant correlations are for mean number of flowers per inflorescence in Upper Seeds in 1990 v 1991 (*Pearson's correlation = 0.81; df=5; p <0.02*) and in 1991 v 1992 (*Pearson's correlation = 0.60; df=12; p <0.02*).

3.4 Trade-offs

The results of the trade-off correlations for 1991 are shown in Table 3.5, and for 1992 in Table 3.6. Individual plants are ranked according to size, as indicated.

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[2] Mean seeds per pod has been based upon the length of unpredated pods as there is a strong positive correlation between pod length and seeds per pod in 1991 (*Pearson's Correlation=0.78; df=37; p <0.001*) and 1992 (*Pearson's Correlation=0.83; df=35; p <0.001*). Pod length was not measured in 1990.

Table 3.5: Trade-off correlations between different aspects of reproductive output, Upper Seeds 1991.

Increasing Plant size	**Plant**	**Pods/head v Seeds/pod**	**Pods/head v Seed weight**	**Seeds/pod v Seed weight**
↑	LU08	ns	-	-
	LU14	ns	Kendall's tau = 0.14, p=0.07	ns
↑	LP15	ns	ns	ns
	LU15	ns	ns	ns
	LU09	ns	-	-
	LU13	ns	ns	ns
↑	LU04	ns	Kendall's tau = 0.21, p <0.02	Pearson's Correlation = -0.34; df=58; p <0.005
	LU05	ns	ns	ns
	LU01	ns	-	-
↑	LU10	ns	ns	

Table 3.6: Trade-off correlations between different aspects of reproductive output, Upper Seeds 1992. Plant size ranking has been done using total flower production.

Increasing Plant size	**Plant**	**Pods/head v Seeds/pod**	**Pods/head v Seed weight**	**Seeds/pod v Seed weight**
↑	LU08	ns	ns	ns
	LU13	ns	ns	ns
↑	LU09	Kendall's tau = 0.19, p <0.05	ns	ns
↑	LU01	ns	-	-

4. Discussion

4.1 Plant size

In general plants from the established sites (Lower Seeds Reserve, Rough Common, The Quarry) were much smaller, and produced far fewer flowers, than plants from the colonising Upper Seeds site, though the largest Lower Seeds Reserve plants overlapped in size the smallest Upper Seeds plants. Possible reasons for the size differences have been discussed in Chapter 2.

4.2 Temporal regulation of flower number

Those plant species which produce inflorescences of variable numbers of flowers can either manipulate the numbers of flowers per inflorescence or the total number of inflorescences, or both. Lloyd *et al.* (1980) found, in the seventeen angiosperm species they studied, that "...variation in inflorescence numbers is an important component of variation in total flower number." How well does this reflect the situation for *L. corniculatus*, a species with variable numbers of flowers per inflorescence? It is apparent from my results that the greatest source of variation in flower number over time is in inflorescence production. Mean numbers of flowers per inflorescence does sometimes vary concurrently with inflorescence number, but by no means on all plants. In his resource manipulation experiment using *Lotus corniculatus* Stephenson (1984) reported that increased resources led to increased flower production, mainly because of more inflorescence production. There was a slightly greater mean number of flowers per inflorescence in the increased resources treatment, but not to the same extent. This seems to be in overall agreement with my findings; *Lotus corniculatus* manipulates its investment in flowers predominantly at the level of the inflorescence, not the individual flower, over time and over changes in resource status.

Mean flower number per inflorescence is correlated with plant size in one year of the study only, but is highly correlated between years, which is understandable as it is a trait which is under genetic control (Jones & Turkington, 1986). Most inflorescences produced are small (Table 3.1); the percentage of one and two flowered inflorescences was *c.* 60% in 1990; *c.* 50% in 1991; and *c.* 70% in 1992[3].

In two of the three years of this study the averaged data for all Upper Seeds' plants showed a marked decline in mean number of flowers per inflorescence over the course of the season (Figure 3.6). I can find no other reference to this phenomenon in the literature, though seed weight is known to decline over the reproductive period in a number of species (Haig & Westoby, 1988a) perhaps in response to a decline in stored nutrients, so it is possible that the decline in mean numbers of flowers per inflorescence reflects a similar situation.

The fact that *Lotus corniculatus* regulates flower production at the level of the inflorescence is difficult to reconcile with accepted resource allocation theory. If we assume that inflorescences are more expensive, in resource terms, to produce than are individual flowers, and that *L. corniculatus* produces far more flower primordia than ever reach anthesis (see Section 1.2), current theory would predict that a strategy of variation in investment at the level of numbers of flowers per inflorescence would be cheaper than variation at the inflorescence level, even if the limiting resource is available meristems. It may be that inflorescences are not as expensive to produce as it first appears; they are mostly green, photosynthesising tissue, and may contribute a large proportion of energy to their own production (Bazzaz & Ackerly, 1992). Nevertheless, there is a cost in terms of mineral nutrients. The control of flower number per inflorescence is probably complex, and whether it is adaptive, perhaps related to pollinator behaviour, or whether it is the result of extrinsic environmental factors over which the plant has no control, is not known. The role of seed predators as possible selective agents of inflorescence size will be considered in Chapter 5.

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[3] The analysis included some different plants in each of the three years, so no conclusions can be drawn about this variability between years.

4.3 Plant size/reproductive output relationships

The relationship between flower production and plant size might be expected to be a positive, linear one for most herbaceous flowering plant species. This is because flower number will be immediately limited by the number of available meristems, and a modular growth form, such as occurs in herbaceous angiosperms, allows meristem production to keep pace with the overall size of the plant. This is the general relationship which has been found for *Lotus corniculatus* at Wytham (Figure 3.7). However, it is apparent from the 1991 results for Lower Seeds Reserve, Rough Common and The Quarry that this relationship need not hold true for all situations at all times. For these sites, this may be indicative of unpredictable, small-scale microsite variation between years; smaller plants such as were found in these sites may be more susceptible than larger individuals to variations in, for example, soil water content and nutrient status, and herbivore activity.

In all of the significant relationships for plant size against flower production (Figure 3.7) the y-axis intercepts are positive. Samson & Werk (1986), in their model of size-dependent reproductive effort, pointed out that such an intercept means that the proportional relationship between reproductive effort and biomass is a negative one; this is the case for proportional reproductive output using flowers, but not using pods, as the measure (Table 3.3). It is apparent that although reproductive output in these plants increases with size, it reduces as a proportional function - larger plants invest a smaller percentage of their carbon allocation to flower production. It seems unlikely, then, that it is ultimately the iteration of new growth modules, and their attendant meristems, which is limiting inflorescence production; if inflorescence production were simply a function of increased potential meristems, then proportional reproductive output should remain the same with increasing plant size. This is in contrast to the findings of Watson & Casper (1984), who believed that morphological constraints may be of great importance in determining levels of reproductive output; specifically, they concluded that meristems can be a limiting resource. Again, such relationships are likely to be species specific.

If it is resources and not relevant tissues which are limiting, why should larger plants put less effort into reproduction, in at least some years? Negative relationships between size and reproductive effort have been found in a number of studies, including that of Ohlson (1988), working with *Saxifraga hirculus* (Saxifragaceae), and Samson & Werk's (1986) reanalysis of previously published data. The theoretical implications of size-dependent reproductive output have been recently explored by a number of workers (Samson & Werk, 1986; Klinkhamer *et al.*, 1992) but we are really no nearer to understanding why it happens. It may be that larger plants require disproportionately more resources for maintenance of tissues, resulting in fewer resources being available for reproduction. This does not appear to have been addressed in the literature, though J. Weiner (cited in Samson & Werk, 1986) has theorised that: "...inherent developmental, structural or physiological constraints may delimit an allometric relationship that results in size-dependent variation in proportional allocation".

Something which I feel should be taken into account in this context is the history of the individual plant. For example, it is possible that the reduced fruit-set observed in Upper Seeds between 1990 and 1991 (Figure 3.11) and reduced flower production between 1991 and 1992 in Upper Seeds and Lower Seeds Reserve (Figure 3.8) were caused by high levels of reproductive output in previous years. These two aspects of reproductive output are not negatively correlated between the three years covered by this study; that is to say, for particular plants, high percentage fruit-set in 1990 did not result in lower fruit-set or flower production in 1991 or 1992. Therefore it is possible that such effects may manifest themselves over long time periods *i.e.* a combined high reproductive output in the year(s) prior to 1990 causing the observed effects in 1991 and 1992. To tease apart such causes and effects in a long-lived plant such as *Lotus corniculatus* one would need to know the complete history of all the individuals under study.

Size of plant is highly correlated with pod production at almost all sites in all years (Table 3.2), even those where plant size is not correlated with flower production. This could be an indication that selective fruit abortion has matched pod production to plant resource status (Stephenson, 1984), assuming that the latter is reflected by plant size (see later discussion on this topic).

The relationship between the proportion of flowers setting fruit and flower production is not a simple linear one (Figures 3.9 & 3.10); fruit-set in a self-incompatible species such as *L. corniculatus* is going to be determined by pollinator activity as well as flower production. This will be explored further in Chapter 4.

The evidence from Table 3.4 is that the seed predators do not exert an influence on seed weight in *Lotus corniculatus*. This finding is in contrast to the work of Ellison & Thompson (1987) previously discussed. It is likely that such phenomena are species specific, but how widespread these effects are is not known at present.

4.4 Trade-offs

The results from the trade-off analyses are not straight forward in either 1991 (Table 3.5) or 1992 (Table 3.6); most individuals show no correlations between the different components of reproductive output and there appears to be no trend regarding plant size. This may be evidence against the hypothesis that the pattern of resource allocation would be dependent on resource status, or it could be that plant size is not a good measure of plant resource status. Almost no work seems to have been done on the latter; the only example that I could find was that of Klinkhamer & de Jong (1993) who found no correlation between the size of individuals of *Cynoglossum officinale* (Boraginaceae) and the concentrations of nitrogen, phosphorous, potassium or magnesium in their leaves, measured either by rootcrown diameter or vegetative biomass. However, neither were there correlations between concentrations of these nutrients and flower production.

There is a possibility that the trade-off ideas which have been taken directly from animal research do not fit so neatly into a plant framework. For a trade-off to occur, it is assumed that a plant is metabolising at its maximum rate given the prevailing internal and environmental constraints; that is, there is no "extra" in reserve. There is growing evidence that for a number of species this is not so (Bazzaz & Ackerly, 1992); the rate of photosynthesis does often increase at the onset of flowering, providing greater reserves of energy for the process of reproduction. For example, Reekie & Bazzaz (1987c) showed that for the grass *Agropyron* (now *Elymus*) *repens*, reproduction was much less costly than might be expected from the quantity of resources allocated to it. Growth of individuals was rarely reduced, and in some cases was actually enhanced. Perhaps better understood is the idea of reduction in resource trade-offs because of photosynthesis by reproductive structures, *e.g.* bracts, stems, petals, developing fruit and ovules (Bazzaz *et al.*, 1979). In real terms, such structures can be considered to be "paying" for their own production and upkeep. It is worth noting that the young fruit and ovules of developing *L. corniculatus* infructescences are green, and are exposed to the light once the petals have abscised (*personal observation*). It seems likely that these structures are photosynthetic and reduce the carbon cost to the parent plant.

Chapter 4: Flowering phenology and floral display.

1. **Introduction: The significance of the floral display**
    - **1.1 Inflorescence architecture**
    - **1.2 Flower number and fruit-set**
    - **1.3 Flowering phenology and fruit-set**
    - **1.4 Mass-flowering and outcrossing in a self-incompatible species - testing Heinrich's Quandary**
    - **1.5 Aims**

2. **Methods**
    - **2.1 Tracking flower production over time**
    - **2.2 Inflorescence tagging**
    - **2.3 Pollinator censusing**
    - **2.4 Weather data**

3. **Results and Discussion**
    - **3.1 The effect of inflorescence size on fruit-set**
    - **3.2 The effect of total flower production on fruit-set**
    - **3.3 Flowering phenology**
    - **3.4 Pollinator activity**
    - **3.5 Testing Heinrich's Quandary**

4. **Conclusion**
    - **4.1 Inflorescence size and fruit-set**
    - **4.2 Flower number and fruit-set**
    - **4.3 Flowering phenology**
    - **4.4 *Lotus corniculatus* and Heinrich's Quandary**

1. Introduction: The significance of the floral display

Within the angiosperms the floral display of species ranges from single flowers, for example some bulbous Liliaceae, to tens of thousands of flowers in the case of tropical forest trees. The size of the floral display presented by an individual flowering plant is a function of four factors.

1. **Constraint** - the evolutionary heritage of a species will be expressed as a phylogenetic/developmental limitation on the type, size and number of flowers and inflorescences produced.

2. **Time of year** - the flowering phenology of the species limits the numbers of flowers displayed at any one time.

3. **Age** - if older plants are larger, then floral display may increase too.

4. **Conditions** - micro-habitat and resource-level factors, among others, will set an upper limit to flower production.

These factors are by no means independent; flowering phenology may be constrained by phylogeny (Kochmer & Handel, 1986) or environment (Rathcke & Lacey, 1985), whilst age and conditions are inextricably linked.

In all populations, at any one time, plants will vary in the size of their floral displays. *Lotus corniculatus* at the Upper Seeds site has a range of total flower production spanning three orders of magnitude (Chapter 3), but over the course of the season, the size of each plant's display changes. A plant's floral display can also be broken down into component parts *i.e.* inflorescences and flowers. We can therefore look at floral display from three perspectives: as a collection of separate parts, as overall flower output, and as a function of changes in numbers of flowers over time.

1.1 Inflorescence architecture

Inflorescence architecture includes flower number, arrangement and phenology, and can affect patterns of fruit-set in several ways (Wyatt, 1982). Inflorescences of *Lotus corniculatus* at Wytham usually have 1 to 5, occasionally up to 9, flowers per inflorescence (see Chapter 3). In this species, is the size of an inflorescence an important determinant of the probability of that inflorescence producing any fruit? There are at least two reasons why the probability of fruit set should increase with larger inflorescences.

1. Pollinators may be differentially attracted to greater numbers of flowers in an inflorescence.

2. Larger inflorescences may be greater resource sinks, garnering relatively more resources, and therefore increasing the probability of a given fruit surviving.

1.2 Flower number and fruit-set

In a self-incompatible, indeterminately flowering species such as *Lotus corniculatus*, the hypothetical relationship between flower production and fruit-set can be examined from the points of view of resource limitation and pollen limitation. Resource limitation of fruit production is a common event, often because indeterminately flowering species initiate many more flowers than they can support as fruit (Stephenson, 1981) and fruit cost more in resources than flowers. "Excess" flowers may function primarily as pollen donors (Bertin, 1988) or reserve ovaries (Ehrlén, 1991) or both. If resource limitation is important in these individuals, then a relationship in which proportional fruit-set decreases with high flower number might be expected, as fruit production reaches a maximum above which a plant's resources cannot support any more. In Chapter 3 we saw that fruit-set in *L. corniculatus* is occasionally 100% in individuals with very few flowers (mostly in Lower Seeds Reserve) but is frequently far less in those individuals producing many flowers.

The traditional view of pollen limitation is that it is a comparatively rare phenomenon compared to resource limitation (Bierzychudek, 1981) but more recent theoretical work has considered pollen and resource limitation to be complex, interacting processes, rather than a simple either-or situation (Haig & Westoby, 1988b; Calvo & Horvitz, 1990). The presence of self pollen on a stigma can effectively limit stigma space for outcross pollen or reduce its rate of germination (Galen *et al.*, 1989). If a larger number of flowers increases the incidence of self pollen on stigmas, because pollinators are remaining on the same plant for a longer period of time, then we might expect a similar relationship to the hypothetical one for resource limitation: with large numbers of flowers, proportional fruit-set decreases.

1.3 Flowering phenology

Initiation of flowering has three primary environmental causes - water availability, temperature and day length - and the effects of these factors in promoting the physiological changes that induce flowering are rather well studied (Rathcke & Lacey, 1985). Less well studied are the environmental effects which shape flowering phenology following the onset of flower production. Photoperiod can be discounted from this as it varies predictably, but few researchers have addressed this issue to find out what is important. One notable exception to this is Lyons & Mully (1992) who found that experimentally increasing the densities of *Nicotiana alata* (Solanaceae) caused plants to have earlier peak flowering and last flowering dates, and reduced the overall synchrony of the plants. Part of my study will be to assess the effects of weather conditions on flowering patterns, looking for consistent trends over the three years.

The paucity of data on the genetic basis of flowering patterns in relation to abiotic and biotic influences has not prevented a number of authors from speculating as to the adaptive, or non-adaptive, function of flowering phenology (*e.g.* Waser, 1979; Augspurger, 1981; Bawa, 1983; Kochmer & Handel, 1986; de Jong *et al.*, 1992; Ollerton & Lack, 1992 [copy in Appendix 1.]). Claims have been made about the importance of intra- and interspecific competition for pollinators (Rathcke & Lacey, 1985; Waser, 1978) effects of seed predators (Augspurger, 1981), requirements for fruit dispersal (Snow, 1965), mutualistic relationships (Waser &

Real, 1979) and environmental factors (Hodgkin & Quinn, 1978) in the evolution of flowering phenology. Though Zimmerman (1988) considers that there is "ample evidence" that flowering time is heritable, the distinction between timing of initiation of flowering and the subsequent pattern of flowering has not been made; a number of studies have looked at the former, few have addressed the latter. There appears to be a strong genetic basis for flowering time in *Lotus corniculatus*; Sandha *et al.* (1977) found that there was a strong heritability for the number of days to flowering, using either seedlings (h^2=84%) or cuttings (h^2=87%), and early and late flowering varieties of birdsfoot trefoil have been bred (Buzzell & Wilsie, 1964).

A number of studies have documented variation in flowering time within plant populations and have attributed this variation as adaptive responses to factors such as: intraspecific competition for pollinators (Rathcke & Lacey, 1985), increased inter-plant pollinator movement (Frankie & Haber, 1983), increased mate availability (Bawa, 1983), dispersion of seed predators (Zimmerman, 1980), variation in intensity and timing of seed predators and seed dispersal (Primack, 1985) and differential selection depending upon the weather (Primack, 1985). This topic has been reviewed and evaluated in Ollerton & Lack (1992) (see copy in Appendix 1) and I will not reiterate them here. Suffice to say that all of the studies so far done have tried to explain flowering asynchrony in adaptive terms, but this does not have to be an automatic assumption. What I wish to do is to quantify variation in flowering time in *Lotus corniculatus*, and then to assess whether it may be adaptive by correlating flowering time and flowering synchrony with fruit-set and seed predation - two factors which will certainly affect individual reproductive output, and may affect individual fitness. Fruit-set is addressed in this chapter, seed predation in Chapter 5. Though this is not a study of natural selection, consistent differences in reproductive output will at least give an indication as to which factors may be important in the evolution of flowering patterns.

1.4 Mass-flowering and outcrossing in a self-incompatible species - testing Heinrich's Quandary

Self-incompatible species which produce large numbers of flowers within a limited period are presented with a dilemma. A large floral display will attract pollinators but will also encourage those pollinators to remain on that plant, thus a significant amount of the pollen moved between flowers will be the plant's own. This could result in stigma clogging as the self-pollen competes for stigma space with the out-cross pollen. Hypothetically, the subsequent reduction in fruit-set could be great. This phenomenon had been recognised for some time by agronomists working with fruit trees, but the first explicit statement of this dilemma that I can trace was published by Heinrich (1975). For the sake of brevity I will therefore refer to this dilemma as "Heinrich's Quandary". Heinrich considered this situation only in relation to trees, but it could apply to any mass-flowering species.

The assumptions of Heinrich's Quandary are as follows.

1. The species must produce a large floral display.

2. The species must be self-incompatible, or suffer inbreeding depression if self-compatible.

3. Pollinator behaviour must be cued to size of floral display such that a greater number of flowers on a plant results in a longer time spent foraging on that plant, resulting in more geitonogamous pollinations.

1.5 Aims

The broad aim of this part of the study is to examine the adaptive nature of the floral display from the point of view of individual inflorescences, total flower output and flowering phenology, and ask what selective and environmental factors may be important. Specifically, the following will be addressed.

1. Does the number of flowers an inflorescence possesses affect the probability of that inflorescence setting fruit?

2. What is the relationship between the number of flowers a plant produces and the percentage of those flowers which set fruit?

3. Within sites and years, how variable are the flowering times of individuals, and is there any evidence to suggest that any variation has an adaptive explanation?

4. What is the relationship between the timing of flower production (flowering phenology) and the timing of fruit-set (fruiting phenology)?

5. Are weather patterns an important determinant of flowering pattern?

6. Does the production of large numbers of flowers over a short period of time result in a reduction in fruit-set *i.e.* does Heinrich's Quandary apply to *Lotus corniculatus* at Wytham?

2. Methods

2.1 Tracking flower production over time

Methods used to collect flower production data have been described in Chapter 3.

The flowering phenologies of individual plants were categorised by date of first flowering, date of peak flowering and synchrony of flowering. The last was calculated using the method of Augspurger (1983), which is a modification of the technique of Primack (1980). The method compares the number of days of flowering overlap of all possible pairs of individual plants, with the index of synchrony for individual plant (i) given by:

$$\left(\frac{1}{n-1}\right)\left(\frac{1}{f_i}\right)\sum_{j=1}^{n} e_{j\neq i}$$

where: e = the number of days both individuals i and j overlap in their flowering, where i and j are different individuals ($j \neq i$).
f_i = the total number of days individual i is flowering.
n = the number of individuals in the sample.

When the flowering time of an individual overlaps completely with all other individuals, the index of synchrony = 1. When there is no overlap in an individual's flowering time, the index of synchrony = 0.

A measure of the overall synchrony of the population can be gained by averaging the individual synchronies; an index of 1 means that all individuals overlap completely, whilst an index of 0 means that there is no flowering overlap between individuals.

The flowers produced at times of low flower production are much less likely to attract pollinators and contribute to the reproductive output of a plant, but could influence the index of synchrony if low flower production were maintained for a number of days. In order to make the index more ecologically significant, those days with low flower production should be excluded from the analysis. However, determining exactly when flower production is too low for successful fruit-set is subjective; Primack's (1980) approach was to include only those days on which his plants had 50% or more of their flowers open, whilst Augspurger (1983) set a flowering threshold of greater than 10% of the total flower production. My approach is different again; in the absence of evidence as to when flower production is so low that few pollinators are attracted and pollen movement limited, I am using a range of flowering thresholds to compare their effect on flowering synchrony. The three thresholds are 0.1%, 10% and 20% of the total flower production of each plant; any days with flower output at or below this level are counted as days when no flowers were produced.

The sample sizes of the two main sites in the three years are shown in Table 4.1.

Table 4.1: Sample sizes for flowering synchrony analysis; all figures are number of plants for which an index of synchrony has been produced.

	Upper Seeds	**Lower Seeds Reserve**
1990	9	10
1991	21	18
1992	18	13

2.2 Inflorescence tagging

Beginning in 1990 individual plants had a sample of their inflorescences tagged with red electrical tape; red was chosen as this colour is unlikely to affect the foraging behaviour of *Bombus* (Barth, 1985). Table 4.2 gives a synopsis of the inflorescence tagging over the three years, detailing numbers of plants and inflorescences tagged and the information recorded.

Table 4.2: Break-down of inflorescence tagging in the three years of the study.

Year	**Location**	**Number of individuals**	**Number of inflorescences tagged**	**Date of tagging**	**Information recorded**
1990	Upper Seeds	15	25 per plant	July	Flowers/inf.
1991	Upper Seeds	6	up to 40 per plant per census	over whole season	date + flowers/inf.
	Lower Seeds	7	+/- all	"	"
	Quarry	5*	all	"	"
	Rough Common	3*	"	"	"
1992	Upper Seeds	15	up to 50 per plant per census	"	"
	Lower Seeds	13*	all	"	"

*total number of marked plants which flowered

An attempt was made to tag the different size classes of inflorescence equally often, though larger inflorescences were comparatively rare (see Chapter 3) and this was sometimes not possible.

The possibility of inflorescence size influencing the probability of fruit-set was also investigated using these tagged inflorescences. For each size of inflorescence (1 to 6 flowers) the number of inflorescences setting at least one fruit was calculated as a percentage of the total number of inflorescences of that size tagged. These data are available for 1990, 1991 and 1992; because of the small sample sizes available for the two largest size classes the data from all Upper Seeds plants involved in the studies were summed. Flower censusing was terminated in early September of each year by the arrival of grazing sheep.

2.3 Pollinator censusing

The activity of *Bombus* spp. on *Lotus corniculatus* in Upper Seeds and Lower Seeds Reserve, and on other plant species at the two sites, was censused in 1991 and 1992.

The activity of pollinators was tracked in 1991 on three of the Upper Seeds plants and three of the Lower Seeds Reserve plants every 6 to 10 days between 4 June & 1 August in Upper Seeds, and between 20 June & 1 August in Lower Seeds Reserve. The number of *Bombus* species visiting each plant and the number of flowers and inflorescences visited, was recorded for 10 or 15 minutes, 3 to 5 times per day. Background *Bombus* activity was measured by recording the number of individuals within a 3m x 3m area, adjacent to the study plants, over a period of 10 minutes, 1 to 3 times per day. This was done on the census days, starting 4 July in Upper Seeds, and 9 July in Lower Seeds Reserve.

In 1992 a different method was used. Between 9 June and 31 August, a census walk was undertaken once or twice a day, every 3 to 7 days. The census walk connected all of the plants under study in the two sites; that in Upper Seeds was approximately 800m in length, while in Lower Seeds Reserve it was *c.* 200m. Every *Bombus* individual encountered within 1m of each side of the walk was identified and recorded, and the plant it was foraging on noted. As each of the marked *L. corniculatus* was approached the number and identity of any foraging bees was recorded, as was the number of flowers visited (up to a maximum of 20) in 1 minute.

Because of difficulties in identifying *Bombus terrestris* and *B. lucorum* to species level whilst censusing, records of these two species have been amalgamated as "*B. terr/luc*", though some records may be of the less common *B. hortorum*. *Bombus pascuorum* is by far the commonest of the small brown species (Prŷs-Jones & Corbet, 1991) and it is safe to conclude that few of the observations of this species will be of the rarer *B. humilis*. Similarly, *B. lapidarius* is far more common than the only other red-tailed black bumblebee, *B. ruderarius*.

2.4 Weather data

Weather data supplied by The University of Oxford's Radcliffe Meteorological Station have been used in this study. This monitoring station is approximately 5km from the Wytham site.

3. Results and Discussion

3.1 The effect of inflorescence size on fruit-set

The number of flowers per inflorescence does not significantly influence the probability of at least one of the flowers in that inflorescence setting fruit (Table 4.3). This has also been found by Kaul (1979) in *Sagittaria brevirostra* (Alismataceae) and by Andersson & Widén (1993) in *Senecio integrifolius* (Compositae).

Table 4.3: Probability of at least one flower from a given inflorescence size setting fruit; as any differences were hypothesised as being directional (*i.e.* increasing probability of fruit-set with increasing inflorescence size) the data have been tested statistically using a Pearson Correlation.

	Number of flowers per inflorescence						
	1	**2**	**3**	**4**	**5**	**6**	**significance**
1990	46%	43%	42%	51%	34%	-	ns
1991	50%	36%	35%	35%	32%	40%	ns
1992	34%	29%	24%	24%	15%	29%	ns

Positive correlations between number of flowers per inflorescence and probability of fruit-set have been reported in *Catalpa speciosa* (Bignoniaceae) by Stephenson (1979) and in *Phlox divaricata* (Polemoniaceae) and *Geranium maculatum* (Geraniaceae) by Willson *et al.* (1979). Those species in which inflorescence size had no effect on fruit-set were pollinated by: *Bombus* spp. (*Lotus corniculatus*), "66 species of insect in four orders" (*Sagittaria brevirostra*) and "bees, hover flies and beetles" (*Senecio integrifolius*); species in which there is a positive effect have pollinators as diverse as: Lepidoptera (*Phlox divaricata*), "large bees" (*Geranium maculatum*) and "large bees and moths" (*Catalpa speciosa*). It would appear that predictable differences in the behaviour of the pollinators of these species cannot fully explain these discrepancies.

If pollinators become fixated on the commonest size of inflorescence, then it might be expected that the most common inflorescence in a population would be the most successful. That is to say, selection would favour plants producing large numbers of the commonest and most successful inflorescence sizes, assuming a genetic basis to inflorescence size. Wyatt (1982) found that for *Aesculus sylvatica* (Hippocastanaceae), the commonest inflorescences were indeed the most successful, though smaller inflorescences were present in greater numbers than expected if selection had acted on inflorescence size in the past. Most of the inflorescences produced by *L. corniculatus* are small, containing one or two flowers (see Chapter 3); this does not reflect the probability of fruit-set. It may be that pollinator selection of small inflorescences is balanced by resource sink strength of large inflorescences, but where does this leave the idea that pollinators are biased towards large inflorescences? Without experimental manipulations the situation must remain unresolved.

3.2 The effect of total flower production on fruit-set

In Lower Seeds Reserve, there is no obvious relationship between total flower production and percentage fruit-set (Figure 4.1). It is perhaps not surprising that stigma clogging and/or resource limitation should not come into play at this site as the plants are producing relatively few flowers.

In 1990 in Upper Seeds there is also no apparent relationship (Figure 4.2) but once again these plants may be producing too few flowers for an effect to be apparent. In 1991 fruit-set may be reduced at high flower production, but data are lacking in the middle of the range. In 1992 there is a negative, linear relationship between flower production and fruit-set (*Pearson's Correlation* = -0.5; $df=13$; $p < 0.03$). Note that Figures 4.1 and 4.2 are identical to Figures 3.9 and 3.10.

The absence of any predictable relationship between flower production and fruit-set in Upper Seeds or Lower Seeds Reserve over the three years suggests that factors other than total flower production are important determinants of fruit-set. Total fruit production is positively correlated with plant size (Chapter 3); perhaps the long-term advantages of a large floral display out-weigh any disadvantage in some years (*e.g.* Upper Seeds in 1992) and it can be considered a bet-hedging strategy, which in a long-lived species such as *L. corniculatus* could be advantageous.

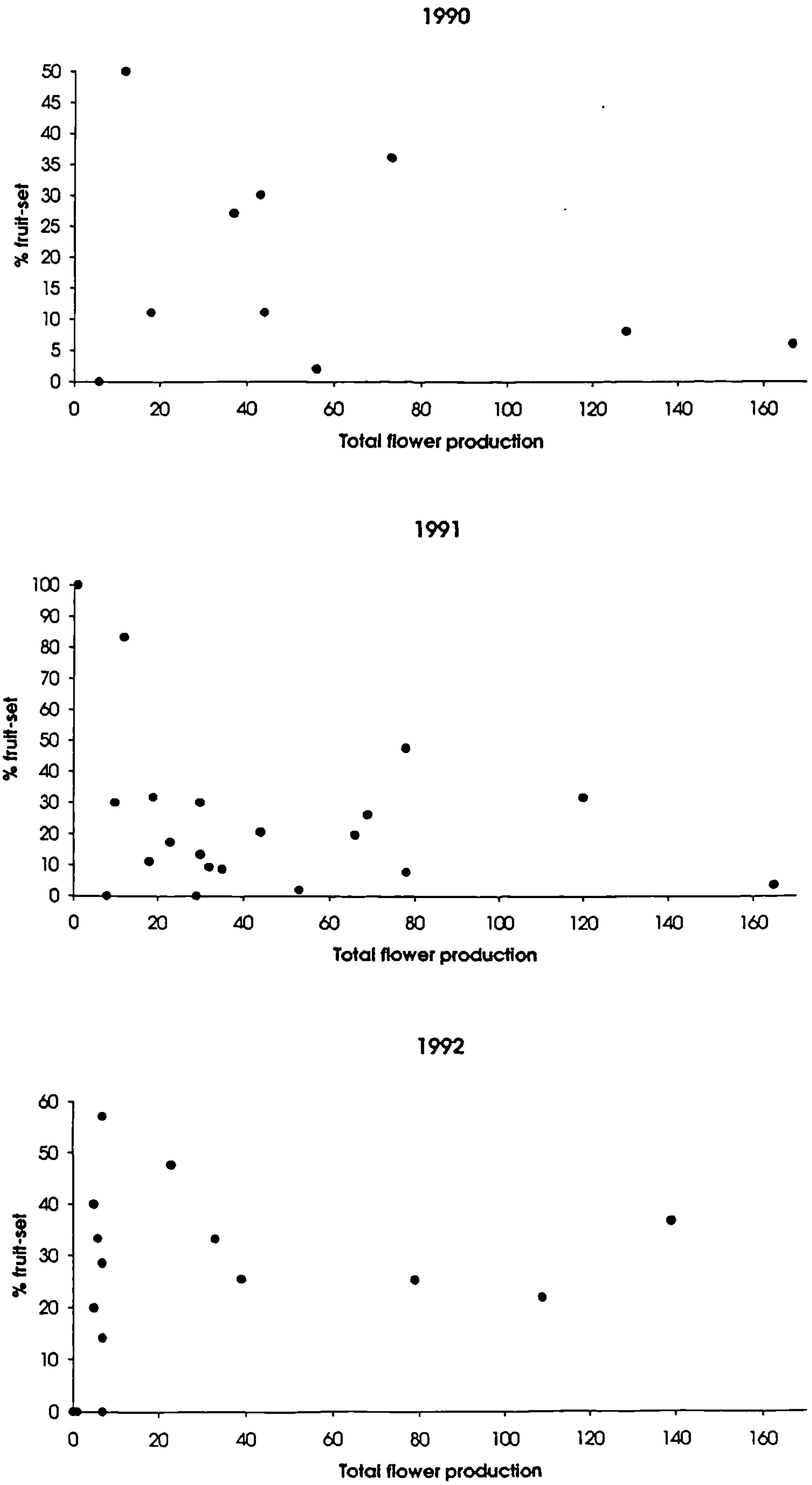

Figure 4.1: The relationship between total flower production and fruit-set in Lower Seeds Reserve in 1990, 1991 and 1992.

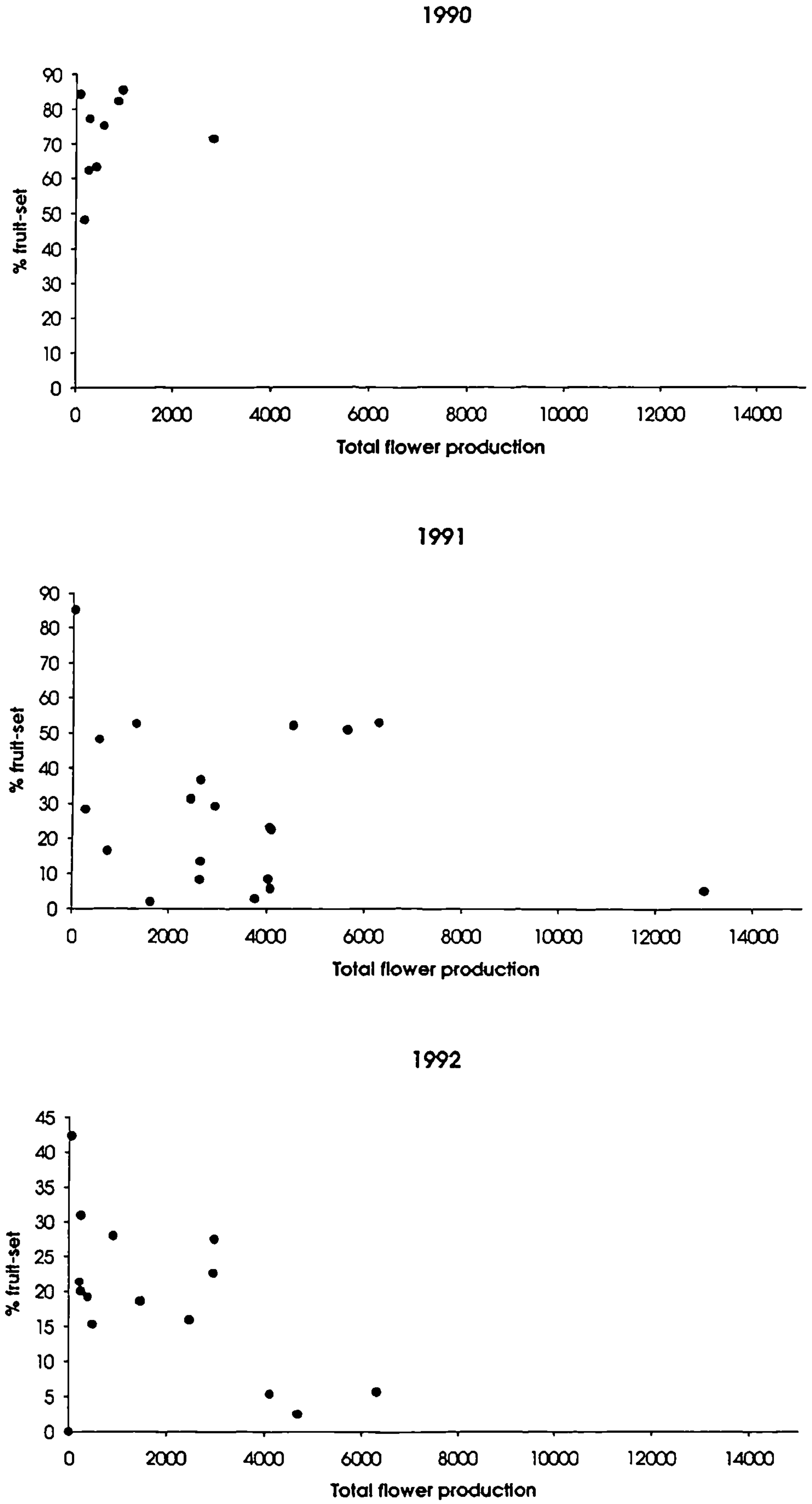

Figure 4.2: The relationship between total flower production and fruit-set in Upper Seeds in 1990, 1991 and 1992.

3.3 Flowering phenology

Too few marked plants flowered in The Quarry and Rough Common to make phenological analysis worthwhile; all of the following results are based on plants in Upper Seeds and Lower Seeds Reserve.

The flowering phenologies of individual plants are variable both within and between years. Examples of the flowering phenologies of the same plants in 1990, 1991 and 1992 illustrate this: five plants from Upper Seeds are shown in Figure 4.3, and four from Lower Seeds Reserve in Figure 4.4. It is worth noting that total flower production is variable between years for the same individuals (see Chapter 3), even to the extent that plant LR07 produced no flowers in 1992 (Figure 4.4).

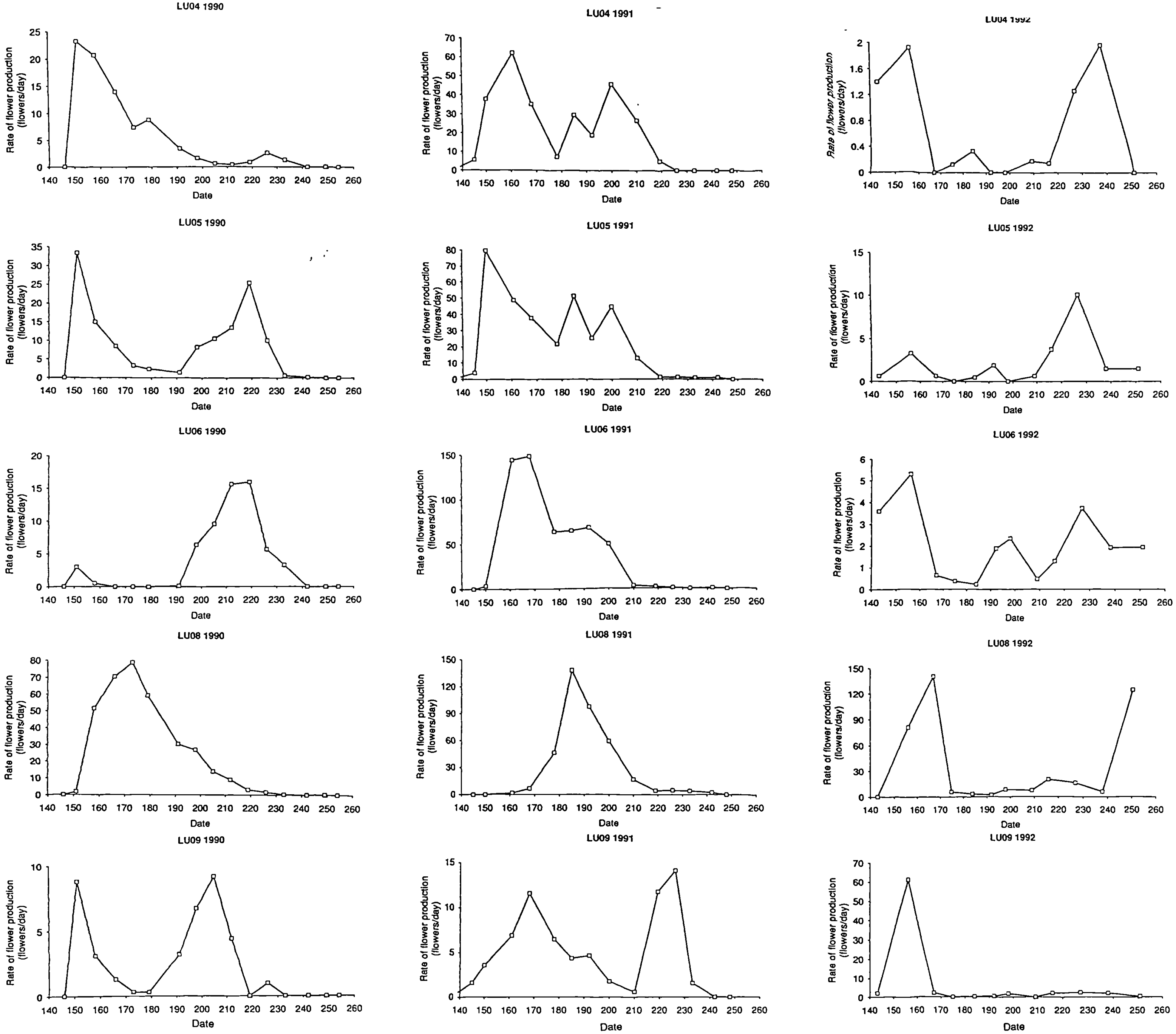

Figure 4.3: Flowering phenologies of five Upper Seeds plants in 1990, 1991 and 1992.

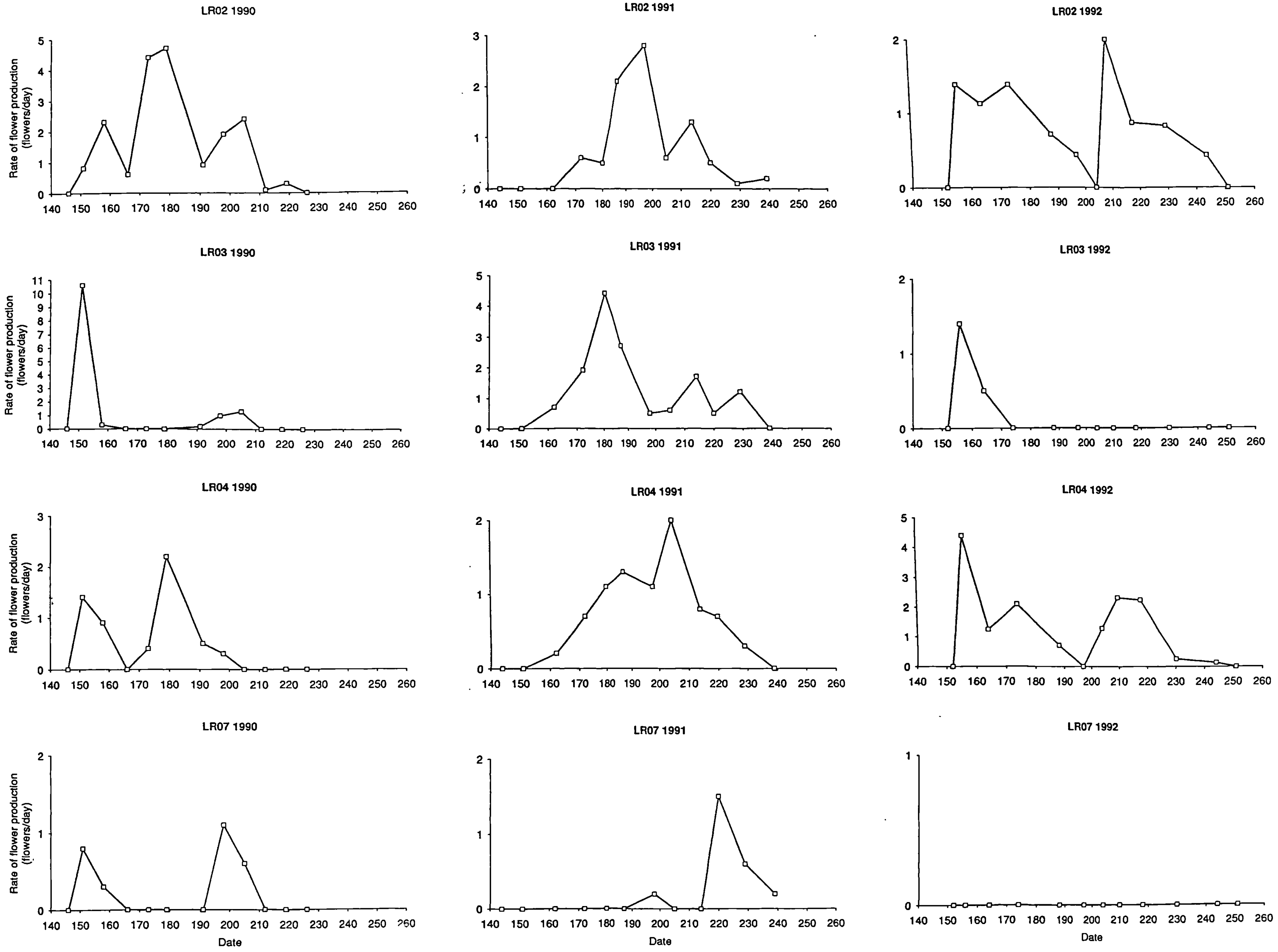

Figure 4.4: Flowering phenologies of four Lower Seeds Reserve plants in 1990, 1991 and 1992.

Some individuals produced more than one flowering peak, a situation also noted by DeGrandi-Hoffman & Collison (1982); they had evidence that the peaks subsequent to the first were the result of a plant having enough resources left to re-initiate flowering. It may be that the first peak is the genetically "predictable" one and that the subsequent peaks are responses to surplus resources, perhaps because of low fruit-set earlier in the season. The only evidence that I have for this is the consistent relationship between timing of first flowering and timing of first peak in Upper Seeds (**1990:** *Pearson's Correlation* $= 0.51$; $df=7$; $p = 0.08$. **1991:** *Pearson's Correlation* $= 0.47$; $df=19$; $p < 0.02$. **1992:** $r^2 = 0.41, p < 0.005$). In Lower Seeds Reserve there is a significant correlation only in 1992($r^2=0.70$, $p < 0.0005$)but the low flower production of plants in this site means that actually deciding when peak flowering occurs is problematical; consequently, the discrepancy between the two sites is not unexpected. In the following tables "peak flowering" refers to the timing of the first peak.

Within years, the timing of first flowering of plants in a site varied by up to 81 days, whilst the first flowering peaks varied by as much as 54 days (Table 4.4).

Table 4.4: Maximum number of days difference in flowering times of plants.

	Maximum difference between plants (days)			
	Upper Seeds		**Lower Seeds Reserve**	
	First	**Peak**	**First**	**Peak**
1990	5	51	10	28
1991	81	49	47	48
1992	24	32	78	54

Between years, the same individuals can vary by 59 days in their timing of first flowering, and 68 days in the timing of their first flowering peaks (Table 4.5).

Table 4.5: Maximum number of days difference in flowering times of individual plants.

	Maximum difference between individual plants			
	Upper Seeds		Lower Seeds Reserve	
	First	Peak	First	Peak
1990 v 1991	32	45	59	48
1991 v 1992	11	38	45	68

Not surprisingly, increasing the flowering threshold in the flowering synchrony analysis results in a lower population synchrony (Table 4.6). In all situations there is a range of individual synchronies; increasing the flowering threshold widens this range. The individual synchronies are highly correlated for the same individuals across flowering thresholds, therefore the values obtained using the 10% threshold have been used in the correlations with fruit-set (see below) and with seed predation (Chapter 5).

Table 4.6: Average index of flowering synchrony for plants in the two main sites.

		Upper Seeds			Lower Seeds Reserve		
Flowering threshold (%)		Mean index of synchrony	Range	95% Confidence Limits	Mean index of synchrony	Range	95% Confidence Limits
0.1	1990	0.83	0.77 - 0.94	0.04	0.76	0.59 - 0.86	0.04
	1991	0.90	0.86 - 1.0	0.02	0.68	0.46 - 0.89	0.06
	1992	0.77	0.72 - 0.84	0.01	0.59	0.54 - 0.68	0.02
10.0	1990	0.67	0.47 - 0.83	0.07	0.66	0.60 - 0.74	0.03
	1991	0.74	0.60 - 0.93	0.04	0.64	0.33 - 0.87	0.08
	1992	0.51	0.31 - 0.71	0.05	0.55	0.47 - 0.67	0.03
20.0	1990	0.52	0.35 - 0.70	0.06	0.59	0.50 - 0.75	0.04
	1991	0.68	0.52 - 0.90	0.004	0.56	0.17 - 0.82	0.08
	1992	0.43	0.16 - 0.66	0.06	0.50	0.40 - 0.65	0.04

In order to test whether the degree of flowering synchrony of an individual affects fruit-set, correlations have been performed between these two components (Table 4.7). Correlations were also performed using date of first flower production; none of these were significant.

Table 4.7: Pearson correlations between flowering synchrony and proportion of fruit-set.

	Upper Seeds	Lower Seeds Reserve
1990	ns	Pearson's Correlation = 0.69; df=8; p <0.02
1991	ns	ns
1992	Pearson's Correlation = -0.46; df=13; p <0.05	ns

There are no consistent relationships between flowering synchrony and fruit-set in either site over the three years of this study. A number of other studies have looked at whether flowering time or flowering synchrony affects plant reproductive success; I have summarised these in Table 4.8. In each case, reproductive success was measured by fruit-, seed- or infructescence-set, and/or seed predation.

Table 4.8: Synopsis of published studies relating flowering time to reproductive success.

		Is reproductive success affected by:	
Species		**Flowering time?**	**Synchrony?**
Petrocoptis grandiflora (Caryophyllaceae)[1]		no	no
Senecio integrifolius (Compositae)[2]		yes	-
Linanthus androsaceus (Polemoniaceae)[3]		no	-
Polemonium foliosissimum (Polemoniaceae)[4]	1977	no	-
	1978	yes	-
	1979	no	-
	1980	no	-
Hybanthus prunifolius (Violaceae)[5]		-	yes
Piper arieianum (Piperaceae)[6]	1981	-	yes
	1982	-	no
Solidago canadensis (Compositae)[7]	1979	yes	-
	1980	yes	-
S. graminifolia[8]		yes	-
S. nemoralis[9]		yes	-
S. juncea[10]		yes	-
Hybanthus prunifolius (Violaceae)[11]		-	no
Turnera panamaensis (Turneraceae)[12]		-	yes
Rinorea sylvatica (Violaceae)[13]		-	no
Psychotria horizontalis (Rubiaceae)[14]		-	no

Table 4.8: (Cont.)

		Is reproductive success affected by:	
Species		Flowering time?	Synchrony?
Erythrina costaricensis var. *panamensis* (Leguminosae)[15]		-	no
Pentagonia macrophylla (Rubiaceae)[16]		-	no
Cynoglossum officianalis (Boraginaceae)[17]		-	yes
Fouquieria splendens (Fouqueriaceae)[18]		yes	-
Silene vulgaris var.*petraea* (Caryophyllaceae)[19]	1988	yes	-
	1989	yes	-
	1990	no	-
Discaria toumatou (Rhamnaceae)[20]	1976	yes	-
	1977	no	-
Leptospermum scoparium (Myrtaceae)[21]	1976	no	-
	1977	yes	-
Dracophyllum spp. (Epacridaceae)[22]	1976	no	-
	1977	no	-

sources: [1]Guitián & Sánchez (1992); [2]Widén (1991); [3]Schmitt (1983); [4]Zimmerman & Gross (1984); [5]Augspurger (1981) [6]Marquis (1988); [7-10]Gross & Werner (1983); [11-16]Augspurger (1983); [17]de Jong & Klinkhamer (1991); [18]Waser (1979); [19]Pettersson (1992); [20-22] Primack (1980).

There is no cross-species consensus on whether flowering time affects reproductive success; some studies have found that flowering time does affect fruit-set and so forth, others have not. This is not so surprising as these kind of interactions might be expected to be species- and site-specific; they do, however, argue against the position that flowering time is always adaptive. Of the five studies which utilise more than one year's data (Primack, 1980; Gross & Werner, 1983; Zimmerman & Gross, 1984; Marquis, 1988; Pettersson, 1992) four discovered between-years differences in the relationship between fruit-set and flowering. This is consistent with my findings for *Lotus corniculatus*; if patterns such as these change between years, this is once again evidence that there may not be the opportunity for natural selection to act through flowering time.

Only two of these studies addressed the *effect of plant size* on *flowering* phenology: Primack (1980) found no correlation between flower production and timing of peak flowering for any of the three New Zealand shrubs that he examined, whilst Schmitt (1983) found that, for the annual *Linanthus androsaceus*, larger plants flowered for a longer period. This is also true of *Lotus corniculatus* in both sites, in all three years: plants with more flowers produce them over a longer period of time (**Upper Seeds - 1990:** *Pearson's Correlation = 0.59; df=7; p <0.05*. **1991:** *Pearson's Correlation = 0.43; df=17; p <0.04*. **1992:** *Pearson's Correlation = 0.49; df=14; p <0.03*. **Lower Seeds Reserve - 1990:** *Pearson's Correlation = 0.69; df=8; p < 0.02*. **1991:** *Pearson's Correlation = 0.76; df=16; p <0.001*. 1992: *Kendall's tau = 0.53, p <0.02*). Having a longer flowering period does not appear to affect the synchrony of an individual, as the only significant correlation between flowering duration and synchrony is in Lower Seeds Reserve in 1990 (*Pearson's Correlation = -0.65; df=8; p <0.03*). The interaction of plant size and flowering synchrony is one which should be considered in future studies - if larger plants have a greater absolute *seed output (as they do in L. corniculatus* - see Chapter 3), plant size might have a much larger effect on individual fitness than other traits such as flowering time, which was the finding of Schmitt (1983).

There is no agreement on exactly which aspect of flowering time is the most important; most studies look at time of first or peak flowering, whilst flowering synchrony is rarely considered. For individuals of a self-incompatible plant such as *Lotus corniculatus*, the flowering patterns of the other individuals in a population must be important - flowering out of synchrony will result in lower fruit-set if there are too few conspecifics to donate pollen. Whether the relative synchrony of an individual will result in selection pressures on flowering time will depend upon two factors: heritability of the trait and differential fitness of individuals. It is well established that there is a genetic basis to date of first flowering in *Lotus corniculatus* (see section 1.3); evidence for the genetic basis of synchrony would be if first flowering time correlated with synchrony - but this relationship is inconsistent (Table 4.9).

Table 4.9: Correlations between first flowering date and synchrony.

	Upper Seeds	**Lower Seeds Reserve**
1990	ns	ns
1991	Kendall's tau = 0.74, p <0.001	Pearson's Correlation = 0.45; df=15; p <0.04
1992	Kendall's tau = -0.38, p <0.04	ns

Not only is the relationship significant in some years but not in others, but the direction of the relationship changes - there is a positive relationship in Upper Seeds in 1991, but a negative one in 1992. Flowering synchrony would appear to be influenced far more by extrinsic, environmental factors rather than intrinsic, genetic ones; it is unlikely, therefore, to be a trait which has evolved. Even if there were a strong heritability to flowering synchrony, the lack of consistent correlation with fruit-set (Table 4.7) means that there is no differential reproductive output being mediated through synchrony, and hence perhaps relaxed selection on flowering time.

Site trends

To examine site flowering trends, the flowering census data of individual plants within sites and years have been converted to proportions of those individuals' total flower production, and then averaged. This is to account for the large differences in flower production between individuals (see Chapter 3). Despite the large error bars (testament to the variability of flowering phenologies within years) there are definite patterns. These patterns are different between years, but are consistent between Upper Seeds and Lower Seeds Reserve.

The role of the weather in shaping flowering phenology

The site flowering phenologies have been compared with weather data for 1990 (Figure 4.5), 1991 (Figure 4.6) and 1992 (Figure 4.7). The following weather data have been used[1].

1. **Rainfall** - daily millimetres of rainfall have been summed between flower census dates to give the cumulative rainfall between censuses.

2. **Sunshine** - daily hours of sunshine have been similarly summed between census dates.

3. **Rainfall + sunshine** - to look for additive effects between these two variables, the data have been summed.

4. **Temperature** - the mean temperature (in Celsius) has been calculated between census dates.

5. **Rainfall + temperature** - the additive effects of these two variables have also been calculated.

Although not clear-cut, these data do show some patterns. In Upper Seeds and Lower Seeds Reserve in 1990 (Figure 4.5) the first flowering peaks correspond to a peak in sunshine. This is also true for Upper Seeds in 1991, but not for Lower Seeds Reserve (Figure 4.6). In 1992, the first flowering peak in Upper Seeds corresponds to a peak of both rainfall and sunshine, but this is not the case in Lower Seeds Reserve (Figure 4.7) There is a consistent lack of correspondence of flowering with temperature in both sites in all years. The additive effects of rainfall plus sunshine and rainfall plus temperature do not appear to explain the observed flowering patterns better than these variables on their own.

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[1] The apparent differences between Upper Seeds and Lower Seeds Reserve in the patterns of the weather data are due to the flowering censuses taking place on different days in these two sites. As the weather data are summed (averaged, in the case of temperature) between census dates, differences do occur. As the object of the exercise is to relate specific patterns of weather and flowering, these differences are immaterial.

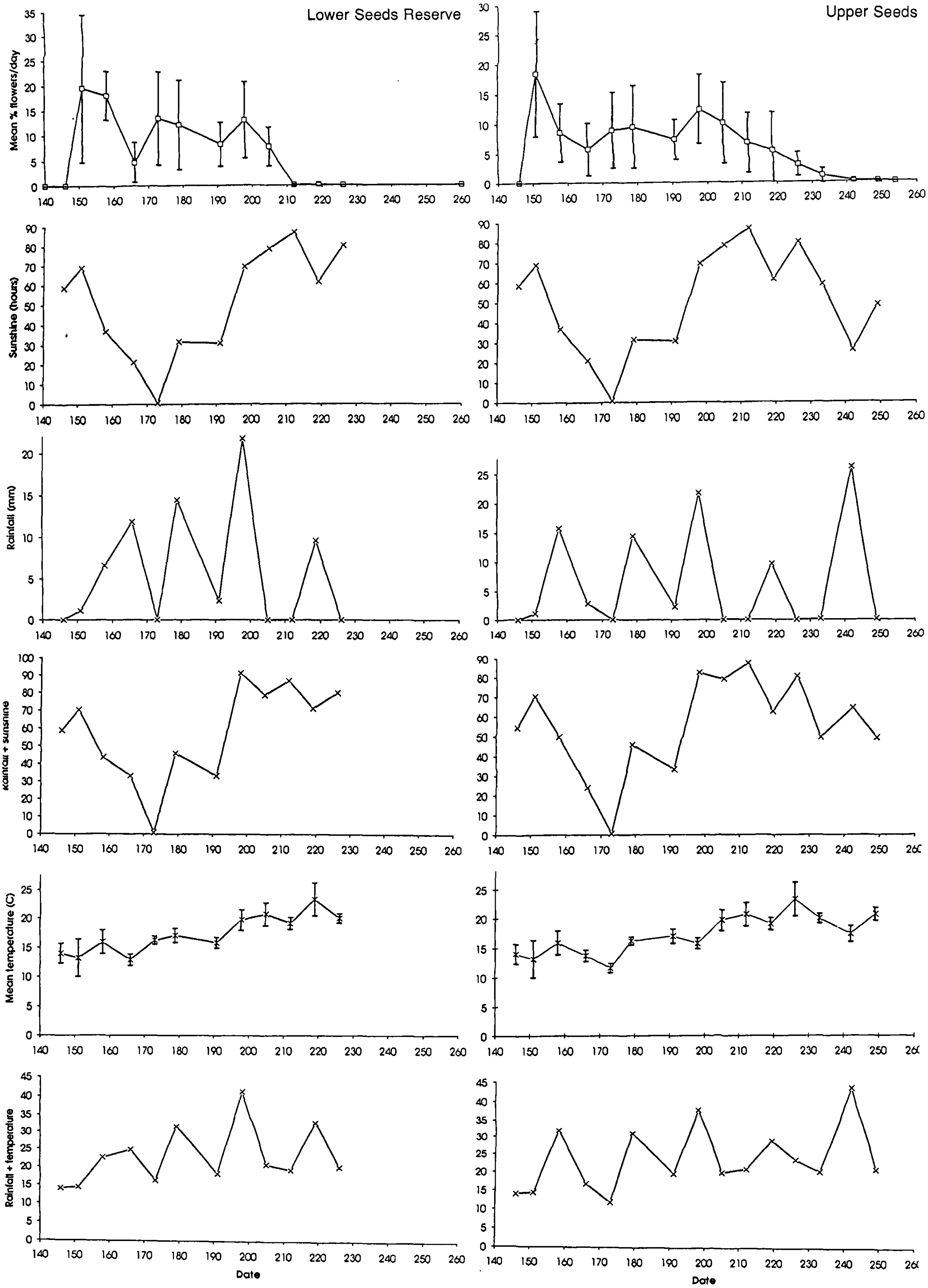

Figure 4.5: The relationship between flowering phenology and weather patterns in Upper Seeds and Lower Seeds Reserve in 1990.

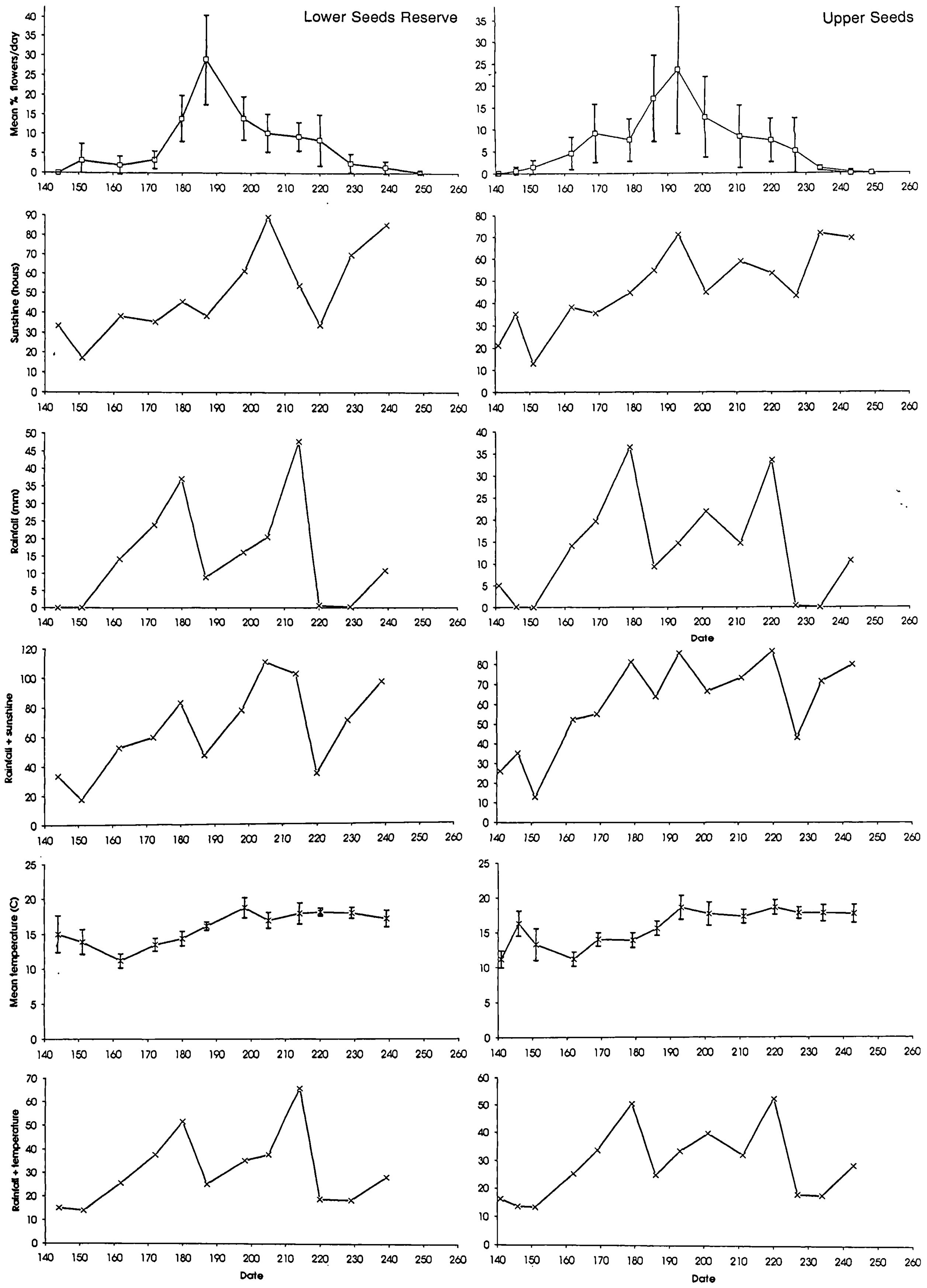

Figure 4.6: The relationship between flowering phenology and weather patterns in Upper Seeds and Lower Seeds Reserve in 1991.

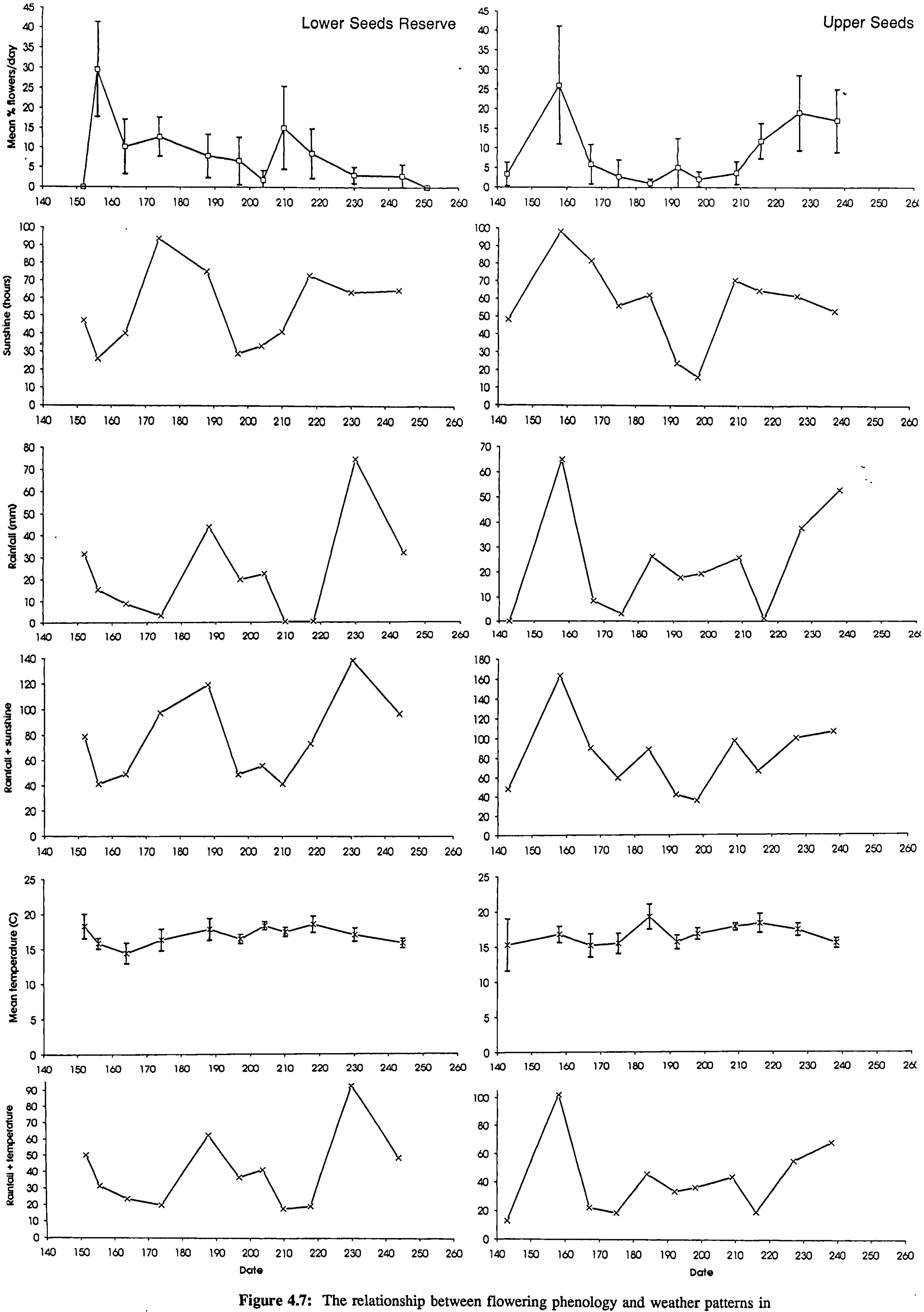

Figure 4.7: The relationship between flowering phenology and weather patterns in Upper Seeds and Lower Seeds Reserve in 1992.

Statistically, one can look for patterns by correlating the weather data with flower production on a given census date. This has been done for all of the weather variables for both sites in each year. Once again, the data are ambiguous. To begin with Upper Seeds: in 1990 there is an almost significant negative relationship between flowering and temperature (*Pearson's Correlation = -0.43; df=13; p=0.053*) but this appears to be an artifact of low flower production at the end of the season coinciding with rising temperatures over the course of the summer (Figure 4.5). In 1991 flowering is positively correlated with sunshine + rainfall (*Pearson's Correlation = 0.50; df=12; p <0.04*); sunshine and rainfall on their own are almost significant (*p=0.08* and *p=0.09* respectively), as is rainfall + temperature (*p=0.06*). Flowering is positively correlated with rainfall in 1992 (*Pearson's Correlation = 0.75; df=9; p <0.004*) and with sunshine (*Pearson's Correlation = 0.52; df=9; p <0.05*). Rainfall + sunshine is also correlated with flowering, though more closely than either of these two variables alone (*Pearson's Correlation = 0.77; df=9; p <0.003*). Temperature alone is not significantly correlated with flowering but temperature + rainfall is (*Pearson's Correlation = 0.78; df=9; p <0.003*).

In Lower Seeds Reserve in 1992, rainfall is almost significantly correlated with flowering, though negatively so (*Pearson's Correlation = -0.45; df=9; p= 0.082*).

In a study of the flowering phenology of *Befaria resinosa* (Ericaceae) Melampy (1987) found a significant positive relationship between monthly flower production and rainfall in the preceding month in one sampling transect, but not for a nearby second transect or an adjacent study plot. Marquis (1988) discovered no correlation between flowering in *Piper arieianum* (Piperaceae) and rainfall in either of two study years. These are the only two reports that I have been able to trace which have looked at the effect of climate in shaping flowering phenology.

There is much evidence of a genetic basis to flower initiation (Rathcke & Lacey, 1985) but this is mostly experimental work done with crop plants and much less is known about the genetics of flowering in natural populations (Pors & Werner, 1989). Once again this work deals with flower initiation rather than flowering pattern; I know of no studies which have examined the latter. It is known that there is a genetic basis to timing of flowering in *L. corniculatus* (see section 1.3). Given this, we might expect that the first flowering times of individuals would be correlated between years; this is true in Upper Seeds for 1991 *versus* 1992 ($r^2=0.72$, $p<0.001$) but not 1990 *versus* 1991. In Lower Seeds Reserve first flowering in neither 1990 *versus* 1991 or 1991 *versus* 1992 were significantly correlated. It would seem that the genetic basis for first flowering is moderated to a high degree by environmental factors. Can the timing of first flowering predict the subsequent pattern of flowering? In Upper Seeds in 1992 there is a significant correlation between timing of first flowering and timing of the first flowering peak ($r^2=0.40$, $p<0.005$); 1991 is also significant (*Pearson's Correlation* = 0.47; $df=19$; $p<0.02$) whilst 1990 is almost significant (*Pearson's Correlation* = 0.51; $df=7$; $p=0.08$). In Lower Seeds Reserve in 1992 first flowering *versus* peak flowering is correlated ($r^2=0.70$, $p<0.0005$), but there are no significant correlations in 1991 or 1990. To a large degree the timing of first flowering does affect the subsequent pattern of flowering, such that early flowerers have an early peak flowering. The exceptions in Lower Seeds Reserve indicate that environmental factors also play a role in this.

3.4 Pollinator activity

There was a generally low *Bombus* activity in Lower Seeds Reserve; in 1991 I observed a total of 19 individuals over the course of censusing, whilst in 1992 I recorded 21. This would appear to reflect the relatively low flower abundance in Lower Seeds Reserve compared to Upper Seeds; the latter is certainly within flying distance of bees from colonies in the former. Consequently I will only be discussing the *Bombus* data from Upper Seeds.

In 1991 and 1992, *Bombus pascuorum* was much the commonest flower visitor to *Lotus corniculatus* (Table 4.10) and probably the most important pollinator.

Table 4.10: Percentages of total recorded visits to *L. corniculatus* by the three *Bombus* spp., and their overall relative abundance.

	B. pascuorum		***"B. terr/luc"***		***B. lapidarius***	
	visiting *Lotus*	**Total abundance**	**visiting *Lotus***	**Total abundance**	**visiting *Lotus***	**Total abundance**
1991	86.1	28.3	5.6	67.9	8.3	3.8
1992	62.1	45.8	19.2	31.0	20.7	23.2

The abundance of *B. pascuorum* and *"B. terr/luc"* on *Lotus corniculatus* does not reflect their overall relative abundance in the two years; *B. pascuorum* forages *L. corniculatus* more often than expected, *"B. terr/luc"* less often. In this respect *Lotus corniculatus* is not alone; the activity of *Bombus* on other plant species at Wytham is similarly decoupled from bee abundance. These data are available for 1992, for five of the commonest plant species in Upper Seeds, including *L. corniculatus* (Figure 4.8). The data have been divided into Early (9 June to 16 July) and Late (22 July to 31 August) season to account for phenological differences in *Bombus* abundance; however, a contingency χ^2 test between Early and Late abundances shows that they are not significantly different ($\chi^2=3.8$; df=2; p=0.15).

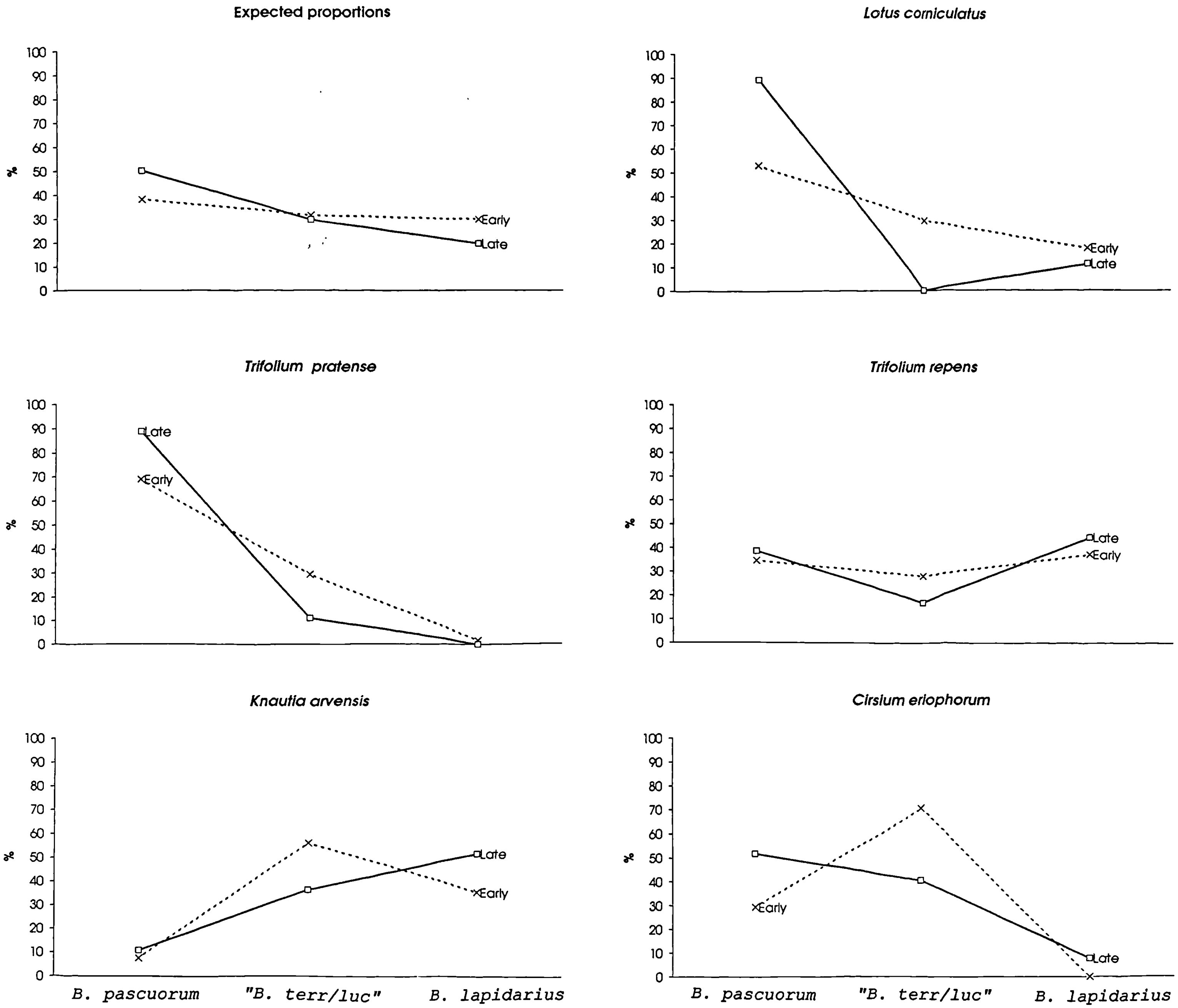

Figure 4.8: Comparison of *Bombus* overall abundance ("expected proportions") and abundance on five plant species; Upper Seeds, early and late season 1992. Line graphs have been used to simplify the interpretation; the abundance data for each species are otherwise unconnected. Abundances are given as a percentage of total bees observed on each species.

In both halves of the season there are discrepancies between the overall abundances of the three bumblebees and their activities on each species. This can be tested statistically using a contingency chi-squared comparing overall *Bombus* abundance with *Bombus* abundance on the plant species (Table 4.11).

Table 4.11: Contingency χ^2 analysis of overall bumblebee abundance *versus* actual abundance on the five plant species; Upper Seeds, 1992; df=2 in all cases.

	Overall abundance *versus*:	
	Early	**Late**
Lotus corniculatus	$\chi^2= 5.6$, $p =0.06$	$\chi^2=42.9$, $p <0.001$
Trifolium repens	$\chi^2= 1.3$, $p =0.5$	$\chi^2=14.9$, $p <0.001$
Trifolium pratense	$\chi^2=34.2$, $p <0.001$	$\chi^2=38.9$, $p <0.001$
Knautia arvensis	$\chi^2=27.9$, $p <0.001$	$\chi^2=40.8$, $p <0.001$
Cirsium eriophorum	$\chi^2=46.1$, $p <0.001$	$\chi^2= 6.7$, $p <0.03$

All of the on-plant abundances are significantly different from the overall abundances, with the exception of *Trifolium repens* in the Early part of the season. In effect, this means that *Bombus* use of forage plants at Wytham is non- random, and that flower resources are being partitioned between the species; this is a situation which has been described many times before (Heinrich, 1976). One way in which flowers become partitioned is because of interspecific differences in tongue length (Brian, 1957; Heinrich, 1976; Ranta & Lundberg, 1980). Short tongued bees cannot forage from flowers with deep corollas, unless they undertake "nectar robbing" *i.e.* biting a hole in the corolla to access the nectar. There is evidence that this may account, in part, for the observed foraging preferences of the 3 bumblebees; for example, the long-tongued *B. pascuorum* virtually monopolises *Trifolium pratense*, which has a corolla length of 12-15mm whereas *Trifolium repens* has a corolla length of 7-10mm and is actively foraged by all three bumblebees (corolla length data from Clapham, Tutin & Moore, 1987).

Lotus corniculatus has a relatively long corolla (10 - 16mm; Clapham, Tutin &

Moore, 1987) and it is also monopolised by *B. pascuorum*. Other studies of *Lotus corniculatus*, however, have found different *Bombus* spp. as the main pollinators. Rasmussen & Brødsgaard (1992), working in Denmark, found that *B. lapidarius* was the sole visitor to *L. corniculatus*, whilst Jones *et al.* (1986), working in Humberside, northern England, recorded *B. lapidarius* and *B. terrestris*. In their study of flower usage by *Bombus* spp. throughout Britain, Fussell & Corbet (1992) only recorded *B. lapidarius* as visiting *L. corniculatus*. Clearly, the role of particular *Bombus* spp. in pollinating *L. corniculatus* is different between localities, perhaps facilitated by the fact that the plant also provides pollen; Rasmussen & Brødsgaard (1992) described *B. lapidarius* as only foraging for pollen at their Danish site.

Differences in tongue length cannot wholly explain this partition of resource use as the relationship is not completely consistent, and the pattern of partitioning changes over the season. For example, *Lotus corniculatus* is a more important species for *Bombus terrestris* earlier in the season than later, despite the fact that the bee's relative abundance hardly changes at all (Figure 4.8). This does not reflect phenological changes on the part of *L. corniculatus*, as flowering intensity does not diminish in the second half of the season, compared with the first (Figure 4.7). An added complication is that intraspecific variation in tongue length allows some workers of short-tongued species to exploit flowers with deep corollas. For example Barrow & Pickard (1984) found that workers of *B. pascuorum* foraging on *L. corniculatus* had significantly longer tongues than those utilising *Trifolium pratense*. Differences in nectar volume and quality may also influence flower use by *Bombus* species (Barrow & Pickard, 1984) as might scent marking of flowers.

Bombus abundance on marked plants

For each census date in 1991 a *Bombus* activity index was calculated as follows:

$$\frac{\text{Number of individuals recorded x Number of flowers visited}}{\text{Number of minutes observed}}$$

Bombus activity in relation to flowering phenology for Upper Seeds is shown in Figure 4.9, and for Lower Seeds Reserve in Figure 4.10 Note that for the purposes of scaling, some of the flowering rates have been divided by ten.

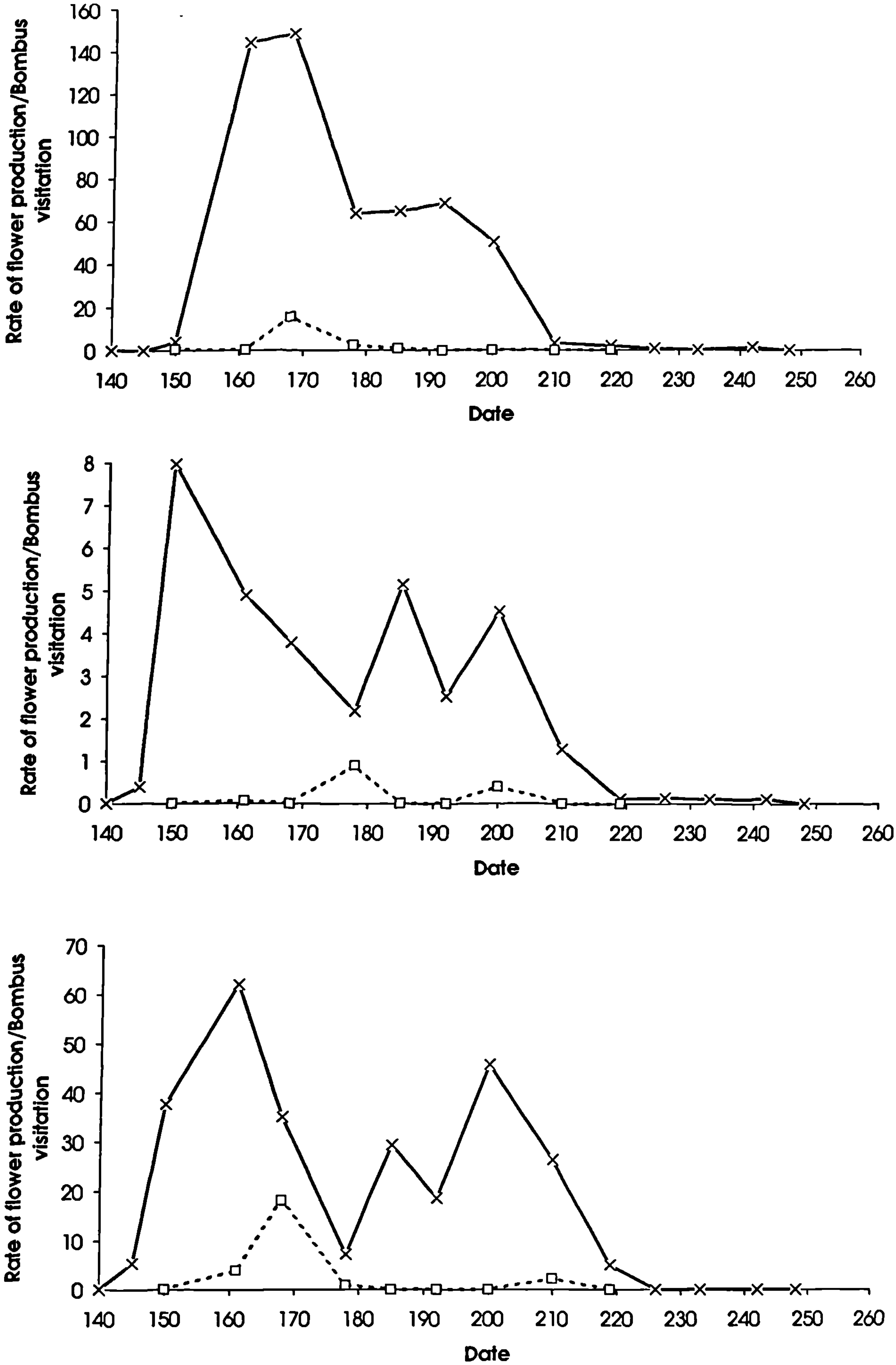

Figure 4.9: *Bombus* activity (- - □ - -) and flowering phenology (--x--), Upper Seeds 1991.

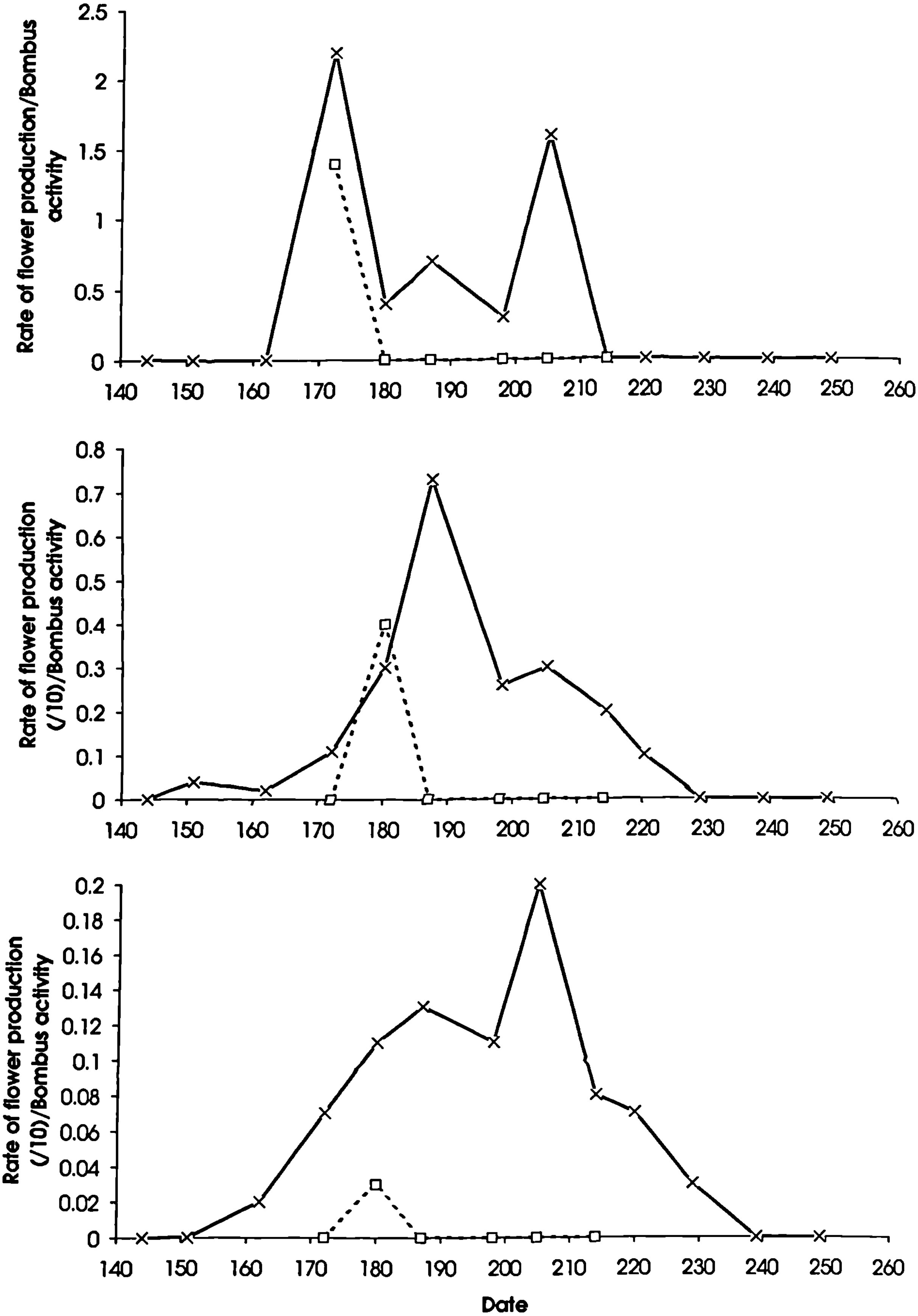

Figure 4.10: *Bombus* activity (- - □ - -) and flowering phenology (--x--), Lower Seeds Reserve 1991.

For the 1992 data *Bombus* activity was calculated as:

$$\frac{\text{Number of flowers visited}}{\text{Number of bees recorded}}$$

So few bees were recorded using the 1992 method that data from the several pollinator censuses nearest to flower censusing days were added together to increase the sample size.

The pattern of *Bombus* activity in relation to flowering phenology and fruit-set for Upper Seeds in 1992 is shown in Figure 4.11. Too few bees were encountered in Lower Seeds Reserve to make this analysis worthwhile.

Peak pollinator activity coincides with peak flowering in five of these graphs and does not coincide with peak flowering in six of them. Those graphs which include fruit-set phenology (Figure 4.11) show peak fruit-set occurring with peak pollinator activity on four out of five occasions. Though there may be other explanations, it is likely that the fifth exception is an artifact of the method used to census pollinators on these plants. One other thing worth noting is that fruit-set generally was low in Upper Seeds *in 1992 (see Chapter 3).*

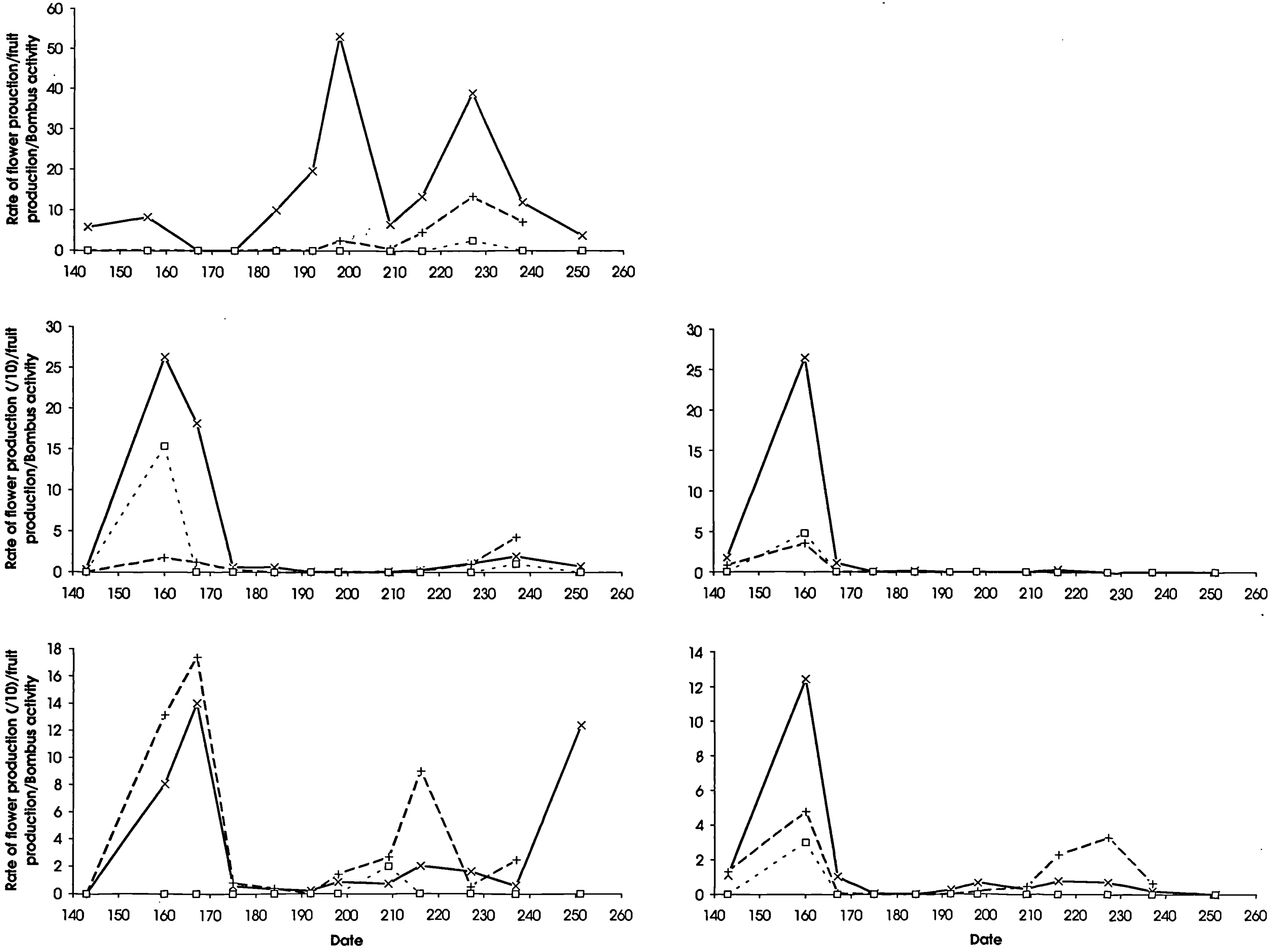

Figure 4.11: *Bombus* activity (- - □ - -), flowering (--X--) and fruit-set (-- -+- - -), Upper Seeds 1992.

There is no evidence from this, then, that *L. corniculatus* plants are timing their flowering to maximise pollination. This is unsurprising given the asynchronous flowering structure of this population. However, in other species *Bombus* are known to preferentially visit larger floral displays (Klinkhamer & de Jong, 1990; Klinkhamer *et al.*, 1989) so it is surprising to find that peak pollinator activity does not always coincide with peak flower production.

3.5 Testing Heinrich's Quandary

One symptom of Heinrich's Quandary would be that peak fruit-set did not coincide with peak flower production on plants with large numbers of flowers. By ranking plants according to their size one can look at whether larger plants show a greater discordance between flowering and fruiting phenologies. This has been done for the Upper Seeds plants in 1991 (Figure 4.12) and 1992 (Figure 4.13). These two figures use the tagged inflorescence data to show which flowers are setting fruit, hence there is no time lag between the flowering and fruiting phenologies.

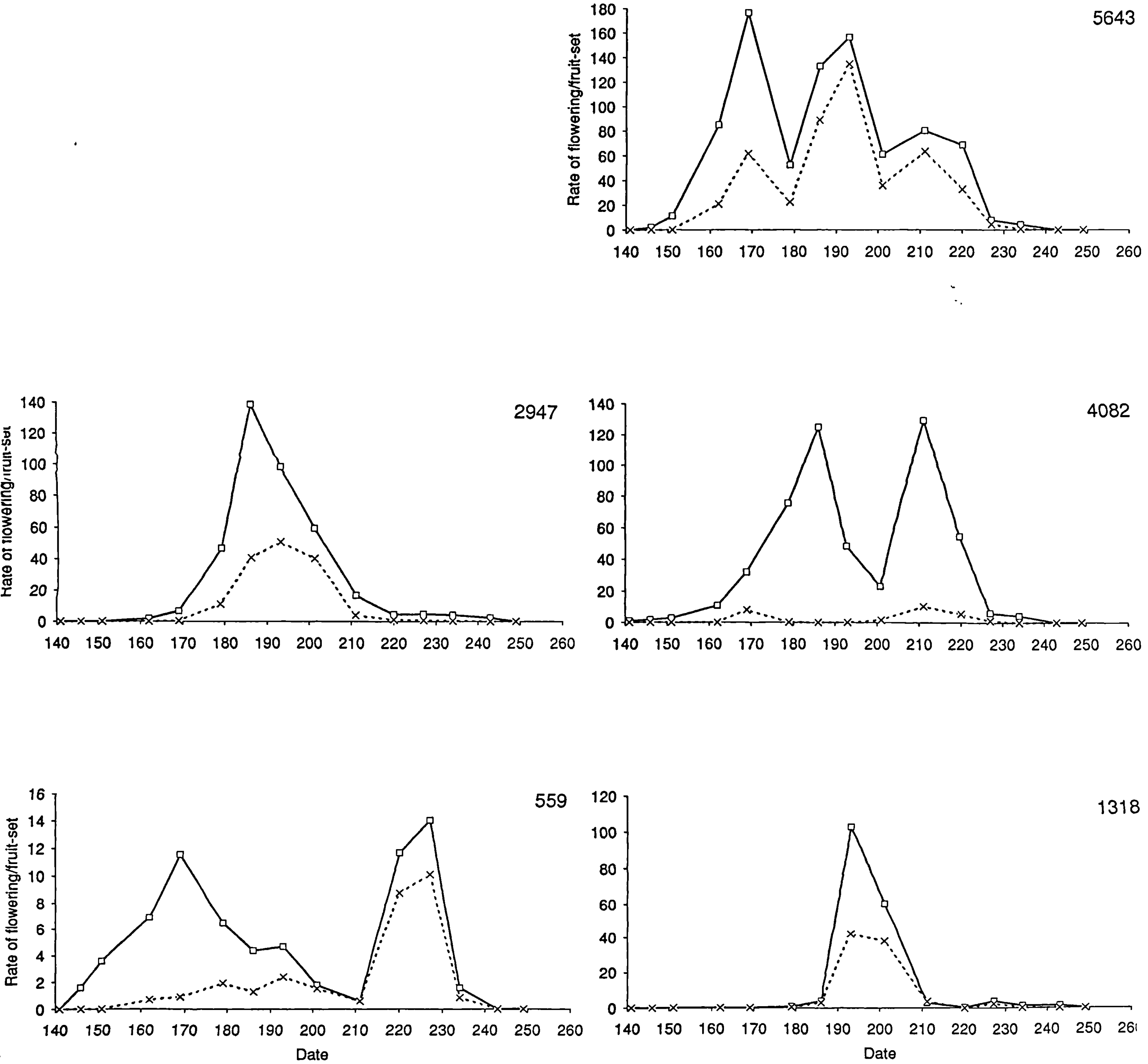

Figure 4.12: Flowering (-□-) and fruiting (-X-) phenologies of Upper Seeds plants in 1991. The plants are arranged in order of total flower production, with the smallest bottom left and the largest top right. The figure in the top corner of each graph is the total flower production for that plant.

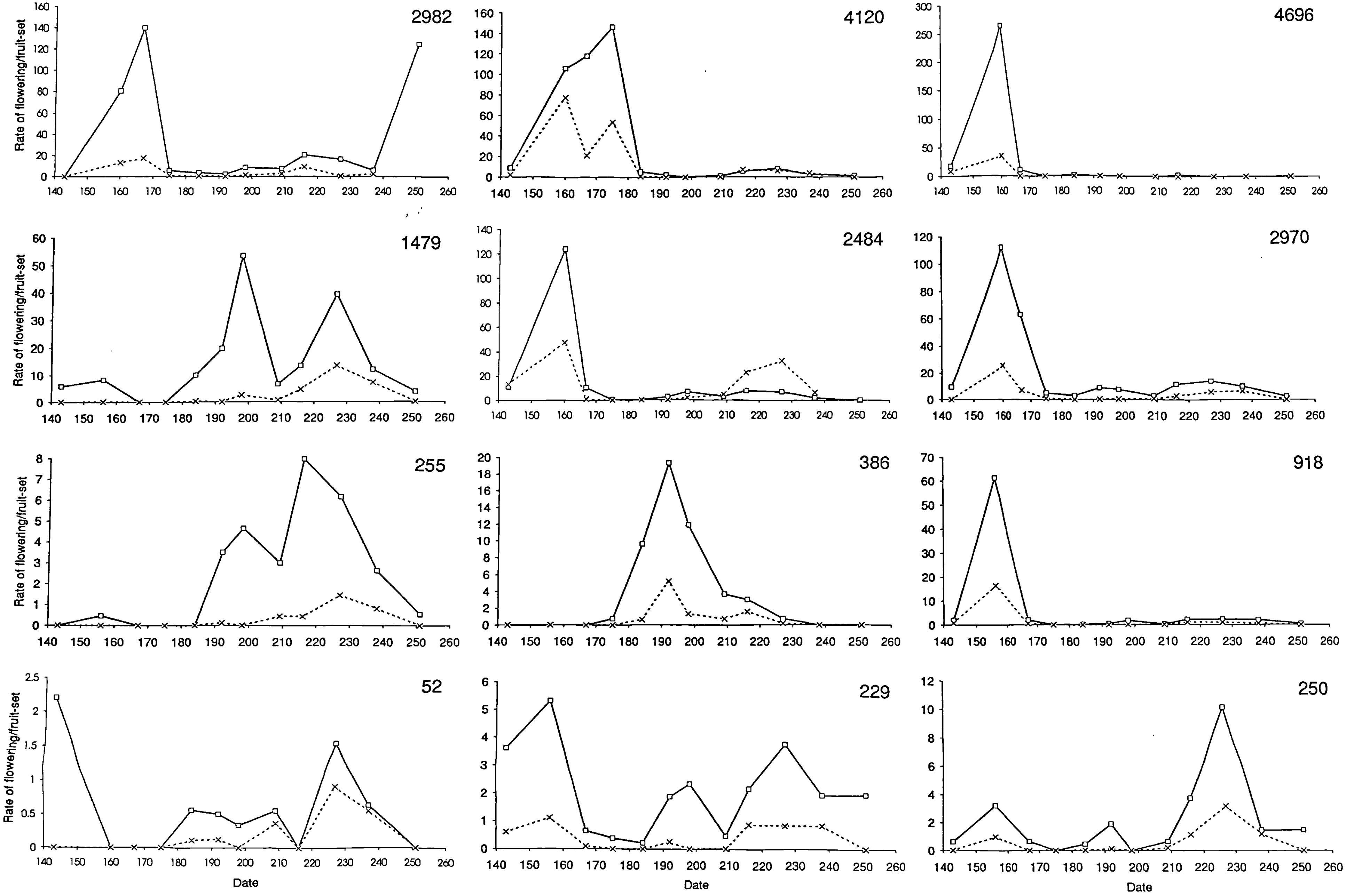

Figure 4.13: Flowering (-□-) and fruiting (-X-) phenologies of Upper Seeds plants in 1992. The plants are arranged in ascending order of total flower production, with the smallest bottom left and the largest top right. The figure in the top corner of each graph is the total flower production for that plant.

There would appear to be no consistent trend between total flower production and timing of peak fruit-set in either 1991 or 1992. Peak fruit-set often does not coincide with peak flowering, but it is apparent that this is not simply a function of size of floral display, as would be expected from Heinrich's Quandary.

Another symptom of Heinrich's Quandary would be if fruit-set were reduced at high levels of flower production. The entire flowering and fruiting data for all Upper Seeds plants has been combined, for 1991 and 1992; the relationship between rate of flower production *versus* rate of fruit production is shown in Figure 4.14 There is no suggestion that in either year rate of fruit production decreases at high levels of flower production; in both 1991 and 1992 the correlations are linear, positive and significant (1991: $r^2=0.68, p<0.001$; 1992: *Kendall's tau=0.45,* $p<0.001$).

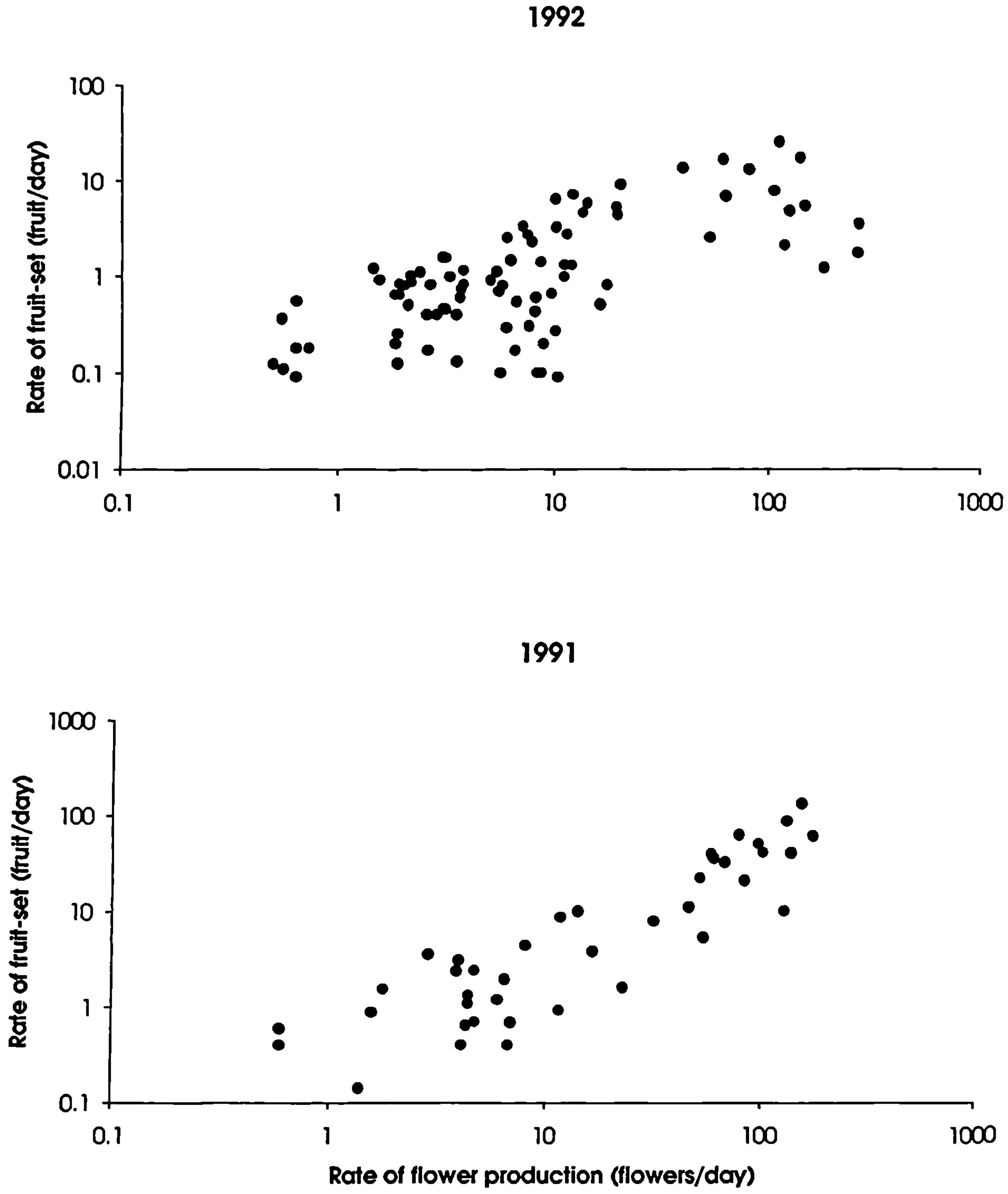

Figure 4.14: Relationship between rate of flowering and rate of fruit-set on each census day for all Upper Seeds plants combined (1991 and 1992). Censuses with zero flowering or zero fruit-set have not been included.

From the data presented here there is no evidence that, at times of high flower production, there is low fruit production on plants of *Lotus corniculatus* in Upper Seeds in 1991 or 1992. Given the assumptions of Heinrich's Quandary already stated, it was expected that the effect would be observed. Could it be that some of the assumptions are wrong in the case of *Lotus corniculatus*? Assumptions 1 and 2 are certainly satisfied by the biology of *L. corniculatus*: as we have seen in Chapter 3, Upper Seeds' plants can produce several thousand flowers over the course of the season, and at times of peak flowering they may be opening at a rate of over 200 per day; also, the self-incompatibility of this species is well established.

Brødsgaard & Rasmussen (1990) performed pollen carryover experiments using fluorescent dye, utilising a *Lotus corniculatus* - *Bombus lapidarius* system. They used an hexagonal array of *L. corniculatus*, and the number of plants visited by the bees on each experimental run ranged from 14 to 64 (mean = 36). Assessments were made of the extent to which dye particles loaded onto the first flower in a sequence were transported to subsequent flowers; the maximum carryover was to a flower 64th in sequence, and the frequency distribution of dye carryover was markedly skewed, with pollen travelling some distance along the sequences (Figure 4.15). Because the number of the variable number of flowers per run, these data have been expressed as percentages. This gives a slightly misleading impression, in that dye carryover in 7 out of 14 flowers is categorised with 20 out of 40 flowers as 50% carryover. Nevertheless, there were never fewer that 14 flowers per run, and usually more (see above) so the conclusion that there is considerable dye carryover by *Bombus lapidarius* on *L. corniculatus* is valid.

If the dye particles are considered analogous to pollen grains, the result of longer pollinator foraging times on *L. corniculatus* would be greater movement of self-pollen between flowers. There is evidence that the presence of self pollen can result in both physical blocking of the stigma (Bertin, 1988) and inhibition of outcross pollen germination (Galen *et al.*, 1989), processes likely to be exacerbated by self pollen carryover of the kind described by Brødsgaard & Rasmussen (1990).

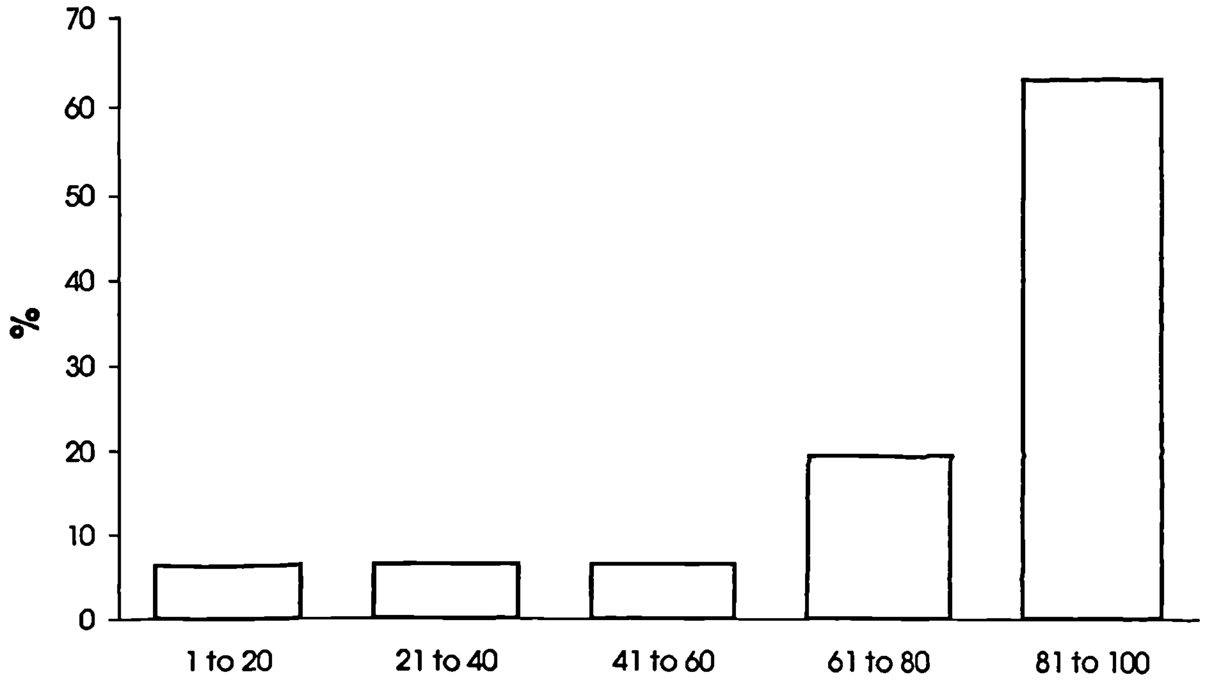

Figure 4.15: Frequency distribution of dye carryover in sequences of *Lotus corniculatus* flowers visited by *Bombus lapidarius*. The categories refer to the percentage of the total flower sequence that the dye was carried-over *i.e.* (number of flowers with dye/total flowers in sequence) x 100. Data from Brødsgaard & Rasmussen (1990).

Could pollinator behaviour be the key to the problem? A number of studies have documented longer visitation times of pollinators on plants with more flowers (for example: Geber, 1985; Schmid-Hempel & Speiser, 1988), including bumblebees (Klinkhamer & de Jong, 1990). Although data on visitation times were not specifically collected in this study, there is a limited amount of incidental data on pollinator movements which could be used. Figure 4.16 shows the relationship between size of floral display and the number of flowers visited by individual *Bombus* in Upper Seeds in 1991 and 1992. Figure 4.17 shows the relationship between floral display and the number of *Bombus* visits per minute, for 1991; the 1992 data were not suitable for analysis in this way.

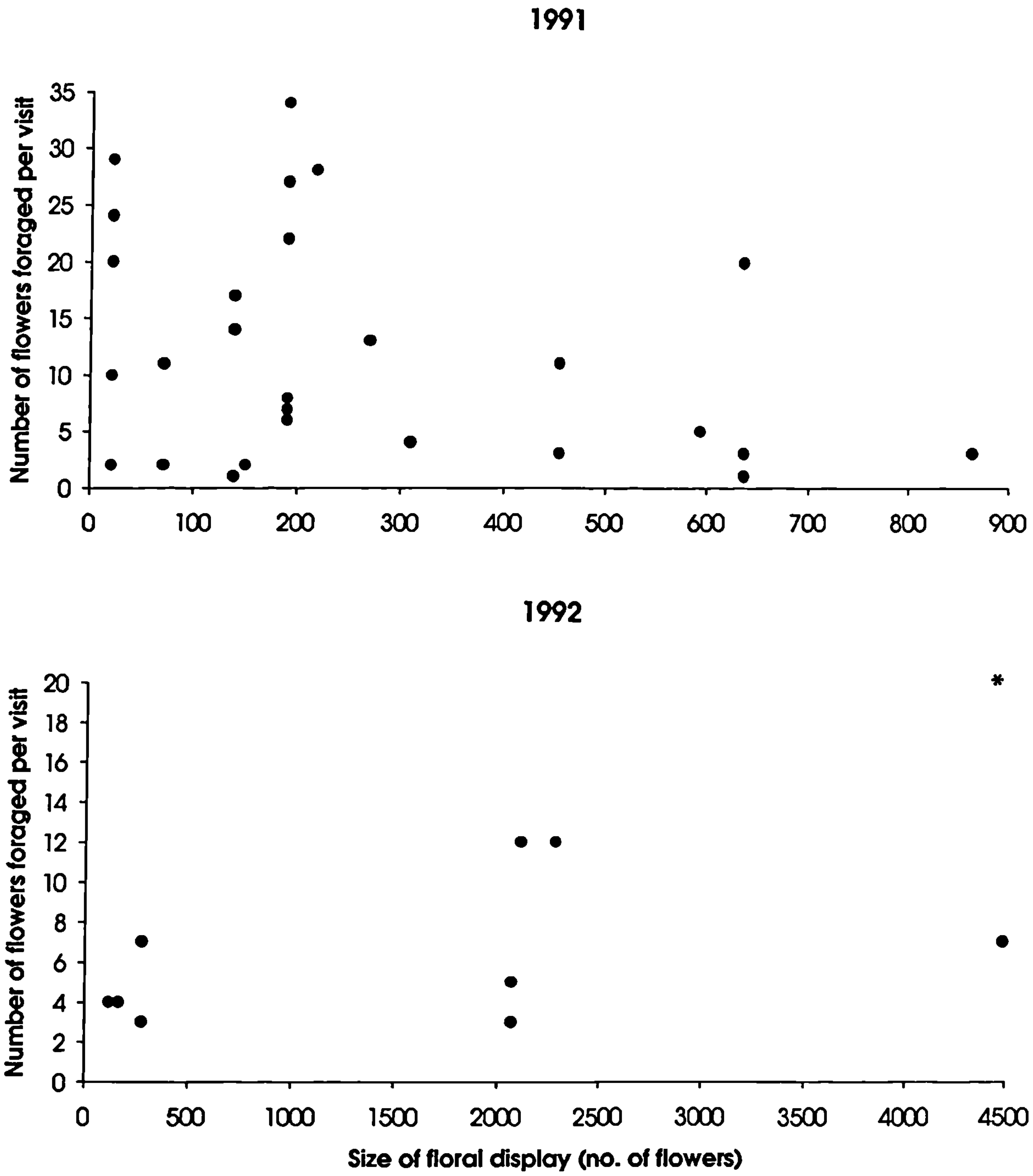

Figure 4.16: The relationship between size of floral display and the total number of flowers foraged per visit by *Bombus*; Upper Seeds 1991 and 1992. In 1992 the data point marked with an asterisk is, in fact, 5 separate data points.

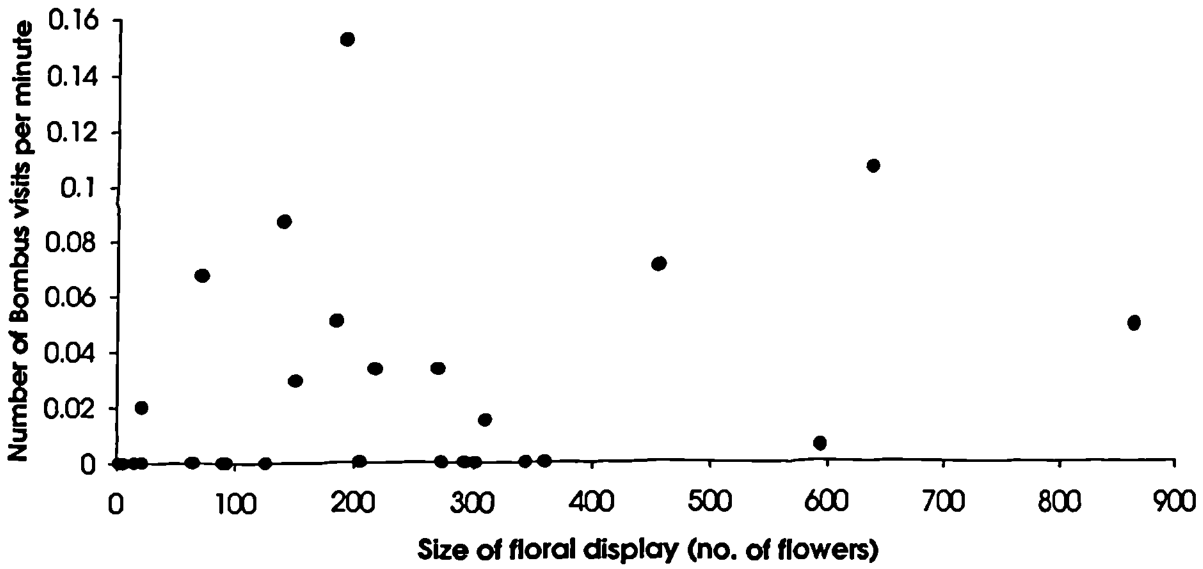

Figure 4.17: The relationship between size of floral display and number of *Bombus* visits per minute; Upper Seeds 1991.

If the pollinators' behaviour was positively affected by the size of floral display, I would expect more flowers to be foraged per visit, and perhaps more *Bombus* visits per minute. The only positive correlation is in the 1992 flowers foraged per visit data (*Pearson's Correlation = 0.82; df=12; p <0.001*). Klinkhamer & de Jong (1990) also found that this latter relationship can vary between years; in two out of three years there was a significant positive relationship between the number of flowers visited by bumblebees and the number of approaches to *Echium vulgare* (Boraginaceae), both of which are correlated with plant attractiveness.

Bumblebees at Wytham do not seem to be responding to the large floral displays of *Lotus corniculatus* in ways which would increase the number of geitonogamous pollinations. Nor do they visit *L. corniculatus* for extended periods of time: if we look at the frequency distribution of number of flowers visited by individual *Bombus* in 1991 and 1992, most visits are of short duration (Figure 4.18).

Despite repeated attempts to extract nectar from *L. corniculatus* using micro-capillary pipettes I was unable to obtain any, a situation which other researchers have also encountered (A.J. Lack, *personal communication,* 1992; Ø. Totland, *personal communication,* 1993). There are published accounts of nectar volume in *Lotus corniculatus,* which is a trait with high heritability (Murrell *et al.*, 1982). For example, Barrow & Pickard (1984) give a figure of 0.45µl per flower, whilst the cultivars assessed by Murrell *et al.* (1982) range for 0.13µl to 0.21µl per flower. To put this in perspective, Barrow & Pickard (1984) also published nectar volumes for *Trifolium repens* of 0.09µl per flower and *T. pratense* of 0.18µl per flower. However, on a per inflorescence basis *L. corniculatus* easily ranks lower than these other legumes, since usually only 1 to 6 flowers are produced per inflorescence (see Chapter 3), whilst the two clovers have over ten times this number. My own lack of success may be due to the fact that it was only ever attempted under field conditions, as were the attempts of A.J. Lack and Ø. Totland, though the former also tried excluding pollinators, with equally little success. Because of removal by pollinators and micro-climatic influences, under field conditions it is standing crop which is being assessed rather than total nectar production. It appears that *Lotus corniculatus* produces very little nectar which in turn discourages pollinators from remaining on plants for too long, reducing the number of geitonogamous pollinations and hence nullifying Heinrich's Quandary.

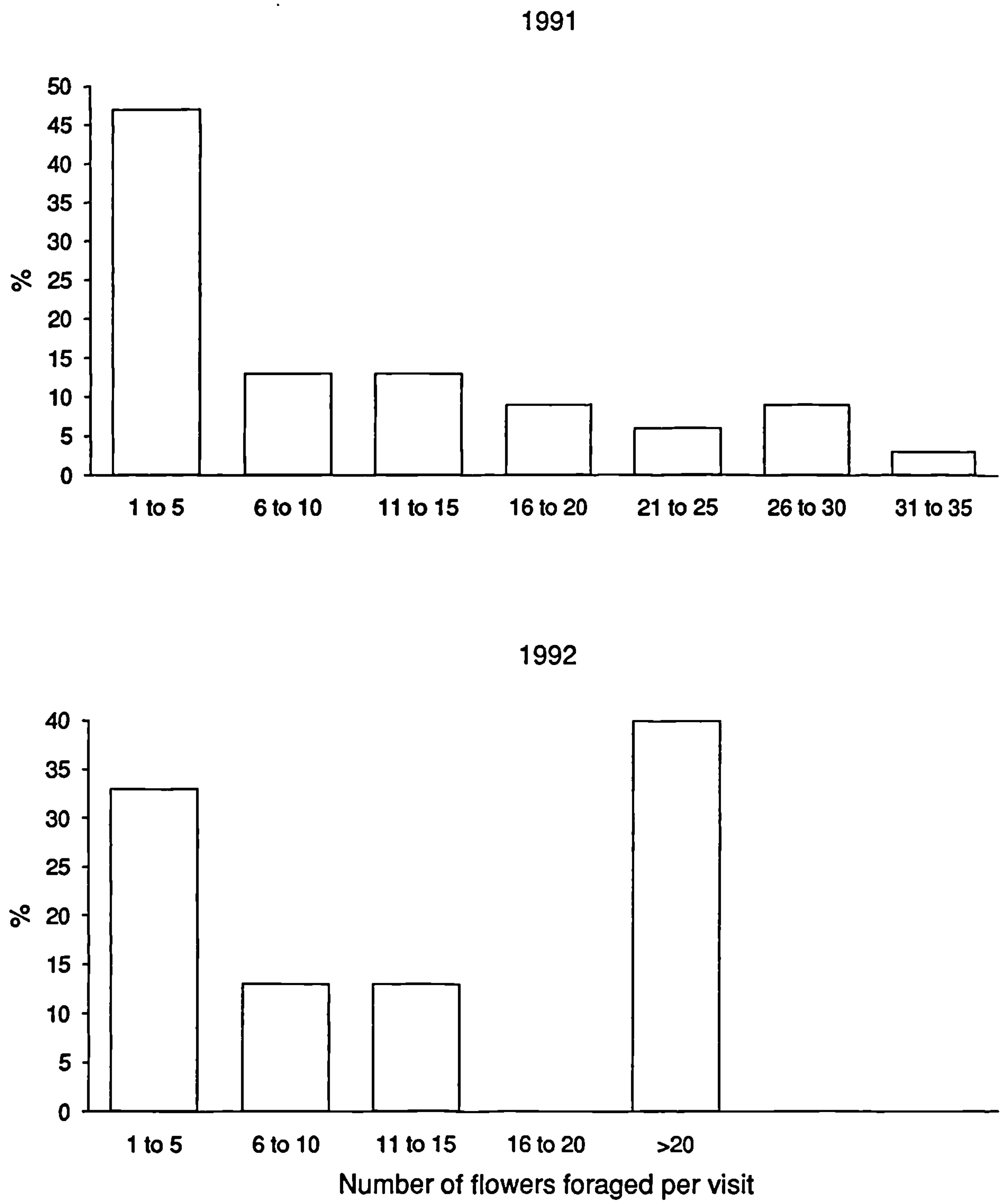

Figure 4.18: Frequency distribution of number of flowers visited by *Bombus* individuals per foraging session, Upper Seeds 1991 and 1992. The data for 1992 are limited by the fact that all visits of more than 20 flowers were grouped together.

4. Conclusion

4.1 Inflorescence size and fruit-set

In *Lotus corniculatus*, the number of flowers an inflorescence possesses does not affect the probability of that inflorescence setting one or more fruit. It may be that *Bombus* spp. are not able to differentiate between different sizes of inflorescences, particularly against a background of the same flowers. This, however, is not borne out by my data; an analysis of the probability of inflorescences with four or more flowers setting fruit indicates that it does not vary with rate of flower production *i.e.* larger inflorescences are apparently not visited more when there are fewer flowers on a plant. From my results in Chapter 3, there are no indications that larger inflorescences garner proportionately more resources, as might be shown by more or heavier seeds in these fruit. Why then is there not a random distribution of inflorescence sizes on plants of this species? The high frequency of small inflorescences cannot be explained by any rational model of resource allocation; it is surely more expensive to initiate a whole new inflorescence than simply to decrease the number of flower primordia aborted in existing inflorescences (see Chapter 3).

As I have already discussed, the body of evidence presented by other authors is inconclusive, and even within species data can be frustratingly inconsistent. Wyatt (1980) found that over two years, one of his two populations of *Asclepias tuberosa* (Asclepiadaceae) showed no difference between the mean size of those inflorescences setting fruit and those that did not. In the other population, in the same two years, inflorescences producing fruit were significantly smaller than those not producing fruit.

Willson & Rathcke (1974) have argued that variation in inflorescence size in *Asclepias syriaca* (Asclepiadaceae) is an adaptive compromise between a minimum number of flowers which is needed for successful fruit-set and a maximum number, above which pollen donation is favoured. If inflorescence size is adaptive in *L. corniculatus*, perhaps selection via pollen donation is favouring individuals of *L. corniculatus* which produce smaller inflorescences, but it is difficult to imagine why smaller inflorescences should be favoured as pollen donors; the question remains unanswered.

4.2 Flower number and fruit-set

The relationship between flower number and fruit-set is unpredictable and, I suspect, subject to influence of weather conditions on pollinator activity. Pollinators will respond almost immediately to a period of cold, wet weather, whereas a plant cannot. Wyatt (1980) has argued that a low relative fruit production (*i.e.* percentage fruit-set) in plants with greater numbers of flowers is not disadvantageous as long as absolute fruit production is higher. Wyatt documented a negative relationship between total flower production and percentage fruit-set (*c.f. L. corniculatus*, Upper Seeds 1992), but a positive relationship between flower production and fruit production (*c.f. L. corniculatus*, most sites, most years: see Chapter 3). Rather than being strategic in any way, the less efficient fruit production of larger plants may be an unavoidable side effect of a high flower output. But in evolutionary terms, an inefficient individual may still be fitter if they produce more offspring.

4.3 Flowering phenology

Flowering synchrony

This study has found no evidence that natural selection has the opportunity to act on flowering time, whether it is either date of first flowering or relative synchrony with conspecifics. There are no differences in reproductive output which are consistent with variation in flowering, at least from a maternal view point; it is conceivable that selection could act on flowering time via differential pollen flow, and hence paternal fitness. As far as I am aware the only study which has looked at this is Melampy (1987) who found that pollen of *Befaria resinosa* (Ericaceae) was dispersed further at times of low flower production in the population; it was not clear whether there were any fitness differences associated with these patterns.

Flowering and the weather

No firm conclusions can be drawn about the influence on flowering pattern of the weather variables that I have looked at. Rainfall and sunshine do affect flowering, but the exact regime changes (and sometimes disappears) between years and sites. Patterns of flowering are probably affected by factors other than weather, for example micro-edaphic and other micro-site characteristics, herbivore activity and plant resource status (see section 3.3). A complete study of how flowering patterns are shaped would need to take all of these into account; as far as I am aware this has never been attempted.

4.4 *Lotus corniculatus* and Heinrich's Quandary

In this study I have looked for evidence that, at times of high flower production in *L. corniculatus*, geitonogamous pollinations result in a reduction in fruit-set. That evidence has not been apparent. If the reason for this is the low nectar production of *L. corniculatus*, can the situation be considered adaptive? It does not have to be; low nectar production in the Upper Seeds' plants may be a side-effect of being large. For most species nectar is largely water and sugar (Heinrich, 1975) and, though plants have been considered "pathological over-producer[s] of carbohydrate" (Harper, 1977), for some species at least there is a cost to be met in its production (Pyke, 1991). A plant might be constrained in its production of nectar if the relationship between green tissue and flowers is such that the demand for nectar exceeds its production. This would have the result of reducing pollinator stop-over time and the resulting self pollinations; it would not be adaptive, rather a fortuitous constraint. It would be instructive to compare nectar volumes per flower in large plants and small plants. Over twenty years ago Heinrich and Raven (1972) pointed out that an optimum nectar supply would be one which kept pollinators interested, but which discouraged long visitation times. It could be that this function is achieved via a non-adaptive process *viz* the inability of large plants to produce per flower nectar volumes which match those of smaller plants.

Heinrich's Quandary has been tested in other species. Stephenson (1982) found that, for the self-incompatible *Catalpa speciosa* (Bignoniaceae) peak fruit-set occurred after peak flowering in both 1979 & 1980. The author took this to mean that the role of pre-peak and peak flowers was to condition pollinators to using the tree's resources, whilst the purpose of the post-peak flowers was to facilitate out-crossing, as the pollinators were more inclined to move between trees at this stage. This study was an interesting test of the Quandary, but can be criticised as only a single individual tree was used; as I have shown in this study, patterns of fruit-set relative to flowering pattern can vary between individuals. Carpenter (1976), working with a mass-flowering species, *Metrosideros collina* (Myrtaceae) found that fruit-set was reduced at peak flowering. Also, fruit-set declined with increasing nectar production in plants which were pollinated by birds + insects, but not in those individuals pollinated by insects alone. Clearly the behaviour of particular pollinators is an important issue here.

As it was first postulated, Heinrich's Quandary dealt with only the fruit-set consequences of increased attractiveness; Klinkhamer & de Jong's (1993b) *theoretical treatment of the "plant's dilemma" addresses* the male component as well. They point out that longer pollinator stays will not only result in more geitonogamous pollinations, but also in less pollen export. There are therefore important fitness considerations from both gender perspectives. Some possible evolutionary resolutions to the Quandary are dealt with by the authors, for example heterostyly, dioecy, herkogamy and dichogamy (see references in Klinkhamer & de Jong, 1993), but their argument always returns to the idea that less nectar equates with increased pollinator movement. From this, the authors set up some testable hypotheses, one of which is that self-incompatible species should experience the strongest selection for reducing geitonogamy, and that such species will have lower nectar rewards than comparable self-compatible ones. If my speculations regarding nectar production are correct, then this position applies to *L. corniculatus*, though as I have already stated I do not believe that it has to be adaptive. In a similar way, Gentry's (1978) observations that pollinator predators congregate on mass-flowering tropical trees, and that their unsuccessful attacks result in more inter-tree movement of pollinators, does not have to be "complexly co-evolved": it may simply be a circumstance of the system, though a fortunate one as far as the plant is concerned.

Robertson's (1992) study revealed that about half of the flower pollinations occurring on large plants of *Myosotis colensoi* (Boraginaceae) were geitonogamous. The author believes this to be fewer than might be expected given the size of the floral display, and attributes it to (a) the fact that the proportion of flowers visited by an individual pollinator decreases with plant size (a phenomenon noted by other researchers [see references in Robertson, 1992] including myself, if the data in Figure 4.16 are expressed as the proportion of flowers foraged per visit) and (b) the high level of carryover of out-cross pollen. In an experimental study of the self-compatible *Malva moschata* (Malvaceae), Crawford (1984) also found a positive relationship between number of flowers open on a plant and the daily rate of self pollinations; a second study of a natural population showed that there was a negative relationship between minimum outcrossing estimate and total flower production. The conclusion from this is that large plants of *M. moschata* produce more selfed seeds than smaller plants. In a recent survey of the literature, de Jong *et al.* (1993). found that geitonogamy is a common, and under-appreciated, phenomenon, and that it usually increases with plant size.

Heinrich's Quandary is a real one - the studies cited above have shown that plants "should" not be too attractive: if they are self-incompatible, the result is reduced fruit-set; if they are self-compatible, offspring quality may be affected. In either case pollen export will decline as pollinators stay longer on a plant. For some species the solution to the Quandary may have been the evolution of herkogamy or dichogamy in their various forms (Klinkhamer & de Jong, 1993), of flower traits which will utilise or modify pollinator behaviour (Robertson, 1992), or of smaller nectar rewards. The solution for other species may not have been evolved: as stated above, I do not believe that Gentry's (1978) pollinator predators hypothesis is necessarily co-evolved, any more than Frankie *et al.*'s (1976) observation that aggressive interactions between solitary bees may have the result of forcing individuals of the same (and other) species to fly to other trees. Finding evidence for the non-adaptive nature of nectar production will require further work; this is to be the subject of an investigation in the near future.

Chapter 5: Effects of seed predation

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1. Introduction

1.1 Seed predation in angiosperms

For a large number of animals seeds represent discrete packages of high protein, high energy food; in the case of *Lotus corniculatus*, Macdonald (1946) recorded a seed protein content of 28.5%, compared with 7.9% for stems and 14% for leaves. Seeds are a rich resource for many vertebrates and invertebrates, and this is reflected in the fact that up to 100% of an individual's seed output can be consumed by seed predators (Crawley, 1992; Sallabanks & Courtney, 1992). Pre-dispersal seed predation has provided evolutionary ecologists with opportunities for studying closely evolved plant-animal interactions. Some of the best examples of close mutualism in plant-insect relationships involve pre-dispersal seed predation, for example *Yucca* spp. (Liliaceae) and yucca moths (Addicott, 1986); *Ficus* spp. (Moraceae) and fig wasps (Janzen, 1979); *Trollius* spp. (Ranunculaceae) and *Chiastocheta* flies (Pellmyr, 1992).

At an ecological scale, the potentially enormous effect that granivory can have on the maternal reproductive output of plants may have important consequences to population demography (Louda, 1982), though in other cases the effects can be insignificant (Andersen, 1989). As Crawley (1992) pointed out, the evidence for and against the importance of seed predation on population flux is "meagre", though seed-limited recruitment may be quite rare in most populations.

At an evolutionary scale, it is thought that seed predation has in the past applied strong selective pressures on some aspects of seed biology. Seed predation has been implicated in mast-fruiting behaviour by certain tree species (Waller, 1993); in delayed synchronous flowering by bamboos (Janzen, 1976b); in timing of fruit abscission (Siemens *et al.*, 1992) and flowering phenology (Augspurger, 1981); and in the evolution of toxic anti-granivore chemicals (Siemens *et al.*, 1992). It is also possible that other, less obvious, plant traits may be subject to selection via seed predation, particularly those which could influence predator behaviour. Flowering phenology is a characteristic which has occasionally been investigated in this way (see Chapter 4). Other potential characteristics which could affect seed predator behaviour are number of flowers per inflorescence, fruit size and seed size. The effect of variation in these characteristics on seed predation has rarely been examined, though it has been hypothesised that selection pressures through the actions of seed predators could be significant in the evolution of floral and reproductive traits in plants (Brody, 1992a,b).

1.2 Pre-dispersal seed predation in *Lotus corniculatus*

Compton (1983) surveyed the insects associated with *Lotus corniculatus*, and described some aspects of their ecology. His study found that the larvae of just three insect species account for the vast majority of pre-dispersal seed predation. These same three insect larvae were found at Wytham. The following accounts of the species' ecologyaretaken from Compton's work.

Cydia compositella (Lepidoptera: Tortricidae)

Mainly a *Trifolium* feeder in Britain, this small moth produces two generations per year; larvae often clear the contents of a developing pod and then move onto another, leaving pods containing nothing but frass and seed debris. Larvae feeding in June or July produce adults in July or August; the second generation stay within the pods until September and overwinter as pupae, emerging as adults in the following June. This species is apparently more common in southern than in northern Britain.

Eurytoma (Bruchophagus) platyptera (Hymenoptera: Chalcidoidea)

The adult female of this chalcid wasp lays a single egg just beneath the testa of an immature seed; Batiste (1967) found that immature seeds, ten to twelve days post-pollination, are preferred. The larvae develop within individual seeds, resulting in a seed which is apparently undamaged, though which may be slightly plumper and paler (*personal observation*) and contains the insect grub. This species is also bivoltine; larvae feeding in July and early August become adult within two weeks; later larvae overwinter within their seeds and pupate in the spring.

Apion loti Kirby (Coleoptera: Curculionidae)

Many *Apion* species are strongly host specific, and *A. loti* is no exception. The species overwinters as an adult, appearing on *L. corniculatus* in May, before the first flowers are produced. They begin mating at this time, and oviposition occurs as soon as the first flowers show themselves. The larvae feed on the contents of the immature pods and pupate in late July/early August. Adults emerge from mid-August onwards, and one generation per year is normal.

1.3 Quantifying seed predation

Quantitative variability in a wide range of traits is a feature of all plant populations. There may be individual differences in the amounts of seed set, susceptibility to herbivory, synthesis of secondary compounds and growth rate. The basis of some of these traits, or the aspects of the plant's biology which lie at the root of them, may have a strong heritability (Weis & Campbell, 1992). If these trait variations then result in differential genotype fitness, natural selection may occur. Seed predation between individuals in a population can be extremely variable, ranging from 0% to 100%; about one quarter of the 134 studies listed by Sallabanks & Courtney (1992) and Crawley (1992) had variations in seed predation that spanned one or two orders of magnitude. For natural selection to occur requires differences to be consistent between years, to result in fitness differentials between individuals, and have a high heritability to the traits governing those differences (Endler, 1986).

Quantifying the amount of seed predation experienced by individual plants, correlating this between years and linking these differences to specific traits are the first steps to evaluating how important seed predation is to individual fitness and ultimately evolution. Seed predation as a factor in natural selection is more straight-forward than pollination as it acts through female function, so the less tractable male function may be ignored. In this study I am not directly measuring selective coefficients mediated by seed predators, but reference will be made to the potential for selection to act in this way.

1.4 Relating seed predation to plant traits

Lotus corniculatus possesses a number of genetically-based traits which may affect seed predation, and perhaps be selected for or against. Timing of flowering, mean number of flowers per inflorescence, mean number of pods per infructescence, mean seed weight and average number of seeds per pod are all characteristics which are under genetic control (Jones & Turkington, 1986), though the results of my study suggest that environmental factors may play a part in determining the exact expression of these traits (see Chapters 3 & 4). In addition, there are other characteristics which may have a weaker genetic component to them, such as flower production and fruit-set (Jones & Turkington, 1986), but are under stronger environmental controls. Distinguishing between environmentally contrived characteristics which would not necessarily affect natural selection, and factors with a strong heritability, is important if one wishes to ask questions about differential seed predation and its effects on fitness through female function. The environmentally determined characteristics are of interest in themselves, and can tell us much about the nature of plant-animal interactions, but they are not likely to influence the evolution of a species.

1.5 Seed predation and flowering phenology

The importance of flowering phenology for pollinator behaviour and subsequent fruit-set has been addressed in Chapter 4. Much less is known about the interaction of seed predators with flowering and/or fruiting phenology, yet a high proportion of seed destruction may conceivably have fitness consequences which are as important as those of seed production. The effect of seed predation on the reproductive output of individuals, relative to their flowering phenologies, will be considered, using the flowering data and methods described in Chapter 4.

1.6 Viability of partially eaten seeds

Whilst assessing the seed pods collected in 1990, it was noted that some seeds were only partially eaten. The larvae of the chalcid wasp (*Eurytoma platyptera*) develop inside individual seeds, whilst those of the moth (*Cydia compositella*) usually consume the entire contents of a pod (Compton, 1983). It therefore seems likely that these part-eaten seeds were the work of the larvae of the weevil (*Apion loti*); whether these are seeds which the larvae did not have time to consume prior to pupation is not known. If seeds of *L. corniculatus* can survive being partially eaten and germinate successfully, this could lead to an over-estimate of the impact of seed predation on an individual plant's seed output. There may also be implications for population dynamics if breaching the seed coat encourages faster germination. An experiment was devised to test the hypothesis that these seeds were still viable.

1.7 Aims

The aims of this part of the project can be framed as a series of questions.

1. What impact does pre-dispersal seed predation have on the reproductive output of individuals of *Lotus corniculatus*?

2. Is there any variation between individuals in the levels of seed predation, and, if so, is this variation consistent between years?

3. Are total flower and pod production, mean number of flowers per inflorescence, mean pods per infructescence, pod size and seed size characteristics which affect seed predation?

4. How does the pattern of flower and fruit production interact with seed predator phenology? Do insect numbers track resource numbers?

5. Can partially eaten seeds survive, and, if so, are the rates of germination affected?

2. Methods

2.1 Quantifying seed predation

Fruits collected in 1990, 1991 and 1992 (methods described in Chapter 3) were assessed in the following ways.

1. Numbers of undamaged seeds in each pod were counted. In 1991 and 1992 the numbers of partially eaten seeds were also counted.

2. Seed predators were identified. In 1990 only presence/absence was scored; in 1991 and 1992, numbers of individuals were counted.

The strong correlation between pod length and seed number (see Chapter 3) was used to estimate the original numbers of seeds in predated pods, using linear regression analysis. From these data, the proportion of each plant's seeds lost to predation was calculated.

In 1990 the moth (*Cydia compositella*) was not encountered, only the frass and other remains of its activity, which were not identified as such at the time. The relative effect of the other two insect species was calculated by scoring the numbers of pods which contained each insect. In 1991 and 1992 a more accurate index of insect effect was calculated by multiplying the mean number of individuals of each of the three species per pod with the proportion of pods predated by that species.

2.2 Interaction of seed predation and plant traits

The data which were collected on a range of individual plant characteristics (see Chapter 3) were correlated with the degree of seed predation experienced by those plants.

2.3 Seed predation and flowering phenology

The fruits collected from the inflorescence tagging experiments in 1991 and 1992 (see Chapter 4) were assessed for numbers of undamaged seeds and identity and number of seed predators, as above. These results were plotted with the flowering phenology data (see also Chapter 4) to show patterns of seed predation over time for individual plants and, by combining the data, for the population. Individual rates of seed predation were correlated with the first flowering date, flowering duration and flowering synchrony data from Chapter 4.

2.4 Seed germination experiment

Seeds which had been obtained from fruit collected in 1991 were categorised into four classes of insect damage.

1. **Undamaged:** the seeds were apparently intact and healthy.

2. **Light damage:** testa partially missing, no significant damage to the cotyledons.

3. **Medium damage:** the cotyledons were damaged, up to a third of the seed missing.

4. **Heavy damage:** more than one third of the cotyledonous tissue was missing.

Twenty seeds of each predation class were sown onto wet filter paper in each of five petri dishes, giving a total of one hundred seeds per class and twenty petri dishes. The petri dishes were arranged systematically within an environmental chamber, in a block 4 x 5, such that dishes containing the same class of seeds were never adjacent horizontally or vertically and were never duplicated within the same vertical column. The temperature of the environmental chamber was maintained at approximately 20°C, with a cycle of 12 hours light and 12 hours dark. The filter paper was watered as necessary with distilled water.

The experiment was begun on 10 February 1992. Each day following the set up of the experiment the seeds were checked for germination. Seeds and seedlings which were attacked by fungus were removed as soon as it was apparent that they were dead.

A second experiment was started on 27 April 1992, using seeds which had been collected in 1990, and which had been stored in plastic specimen tubes at room temperature. The details of the second experiment were otherwise identical to the first.

It had been intended that the seedlings resulting from the two experiments be grown on to see whether seed damage affected subsequent plant growth. A failure of the thermostat controlling the environmental chamber, which effectively baked the seedlings, meant that this was not possible for the first experiment. As soon as the seedlings from the second experimental run were large enough to handle, they were planted into John Innes no. 1 compost in separate 5cm x 5cm compartments of a 6 x 4 compartment seedling tray. The young plants were grown under common (windowsill) conditions for 102 days. Their above ground parts were then harvested, dried in a hot air oven at 100°C for 24 hours[1], and the dry weight recorded.

The trays of, now cropped, seedlings were grown outside over winter and, in March 1993, assessed for survivorship.

3. Results

3.1 Quantification of seed predation

The degree of seed predation was variable between individuals and between years (Figure 5.1); there are no consistent trends between 1991 and 1992. The 100% predation suffered by a number of Lower Seeds Reserve plants may be due to their producing very small numbers of fruits. In 1990 pod length was not measured and

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[1] Subsequent drying of a sample of these seedlings at the same temperature for a further 48 hours did not significantly reduce their dry weights.

therefore an estimate of total seed predation cannot be made. There is a positive correlation between the proportion of pods on a plant with seed predators in them (which was assessed in 1990) and the proportion of seeds predated (**1991:** Upper Seeds: $r^2 = 0.53, p < 0.001$; Lower Seeds: $r^2 = 0.2, p < 0.03$. **1992:** Upper Seeds: $r^2 = 0.56, p < 0.005$; Lower Seeds: $r^2 = 0.32, p < 0.04$). The seed predation correlations in 1990 have therefore been calculated using the proportion of pods with predators in them.

There are no significant between-years correlations for proportion of seeds or pods predated on an individual in either Upper Seeds or Lower Seeds Reserve. Heavily predated individuals in one year are not more likely to be heavily predated the next year.

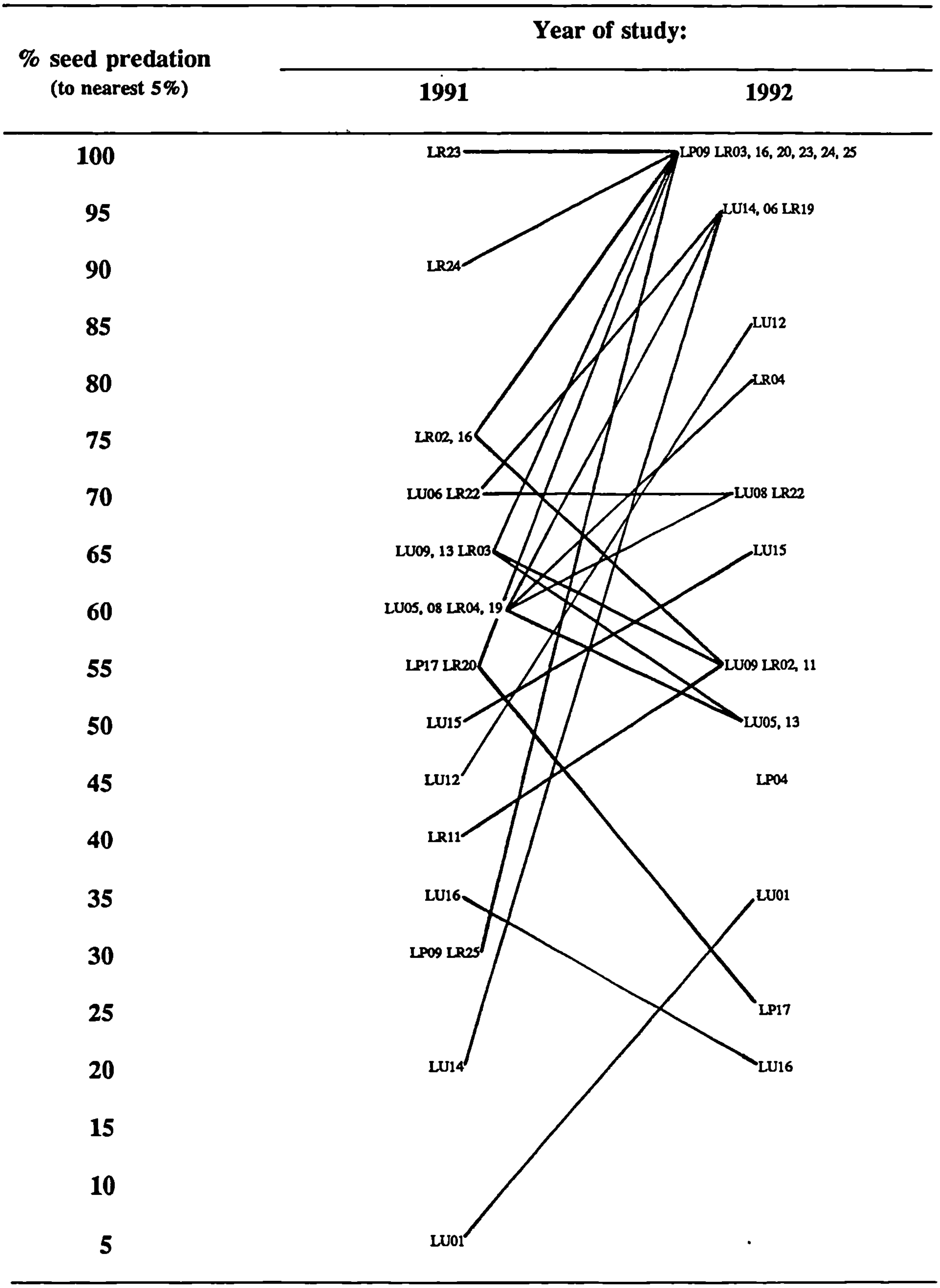

Figure 5.1: Percentage seed predation suffered by plants in Upper Seeds and Lower Seeds Reserve (1991 and 1992). LU.. = Upper Seeds; LR.. = Lower Seeds Reserve.

3.2 The effects of the three seed predators

Individual plants varied in the relative proportions of the three seed predators which attacked their pods in both Upper Seeds (Table 5.1) and Lower Seeds Reserve (Table 5.2). There is no significant between-years correlation for predation by each of the insects; for example, individuals which were heavily infested with weevils in 1990 were not more likely to be infested by them in 1991.

Table 5.1: Range of proportions of the three seed predators found on individual Upper Seeds plants over the three years.

	Range of proportions (%) of each insect		
	Weevil **(*A. loti*)**	**Wasp** **(*E. platyptera*)**	**Moth** **(*C. compositella*)**
1990	14.3 - 87.0	13.0 - 85.7	-
1991	0.03 - 66.7	33.3 - 99.9	0.0 - 1.1
1992	30.0 - 85.7	14.3 - 70.0	0.0 - 6.5

Table 5.2: Range of proportions of the three seed predators found on individual Lower Seeds Reserve plants over the three years.

	Range of proportions (%) of each insect		
	Weevil **(*A. loti*)**	**Wasp** **(*E. platyptera*)**	**Moth** **(*C. compositella*)**
1990	50.0 - 100	0.0 - 50.0	-
1991	0.0 - 100	0.0 - 100	0.0 - 14.3
1992	0.0 - 100	0.0 - 100	0.0 - 2.3

The numbers of moths encountered were so small that they will not be included in the following analyses.

On a population level, there were marked differences in the relative numbers per plant of the weevil and the wasp (Figure 5.2) over the three years. For example, in Upper Seeds in 1991 weevil numbers were much lower, and wasp numbers much higher, compared with the other two years.

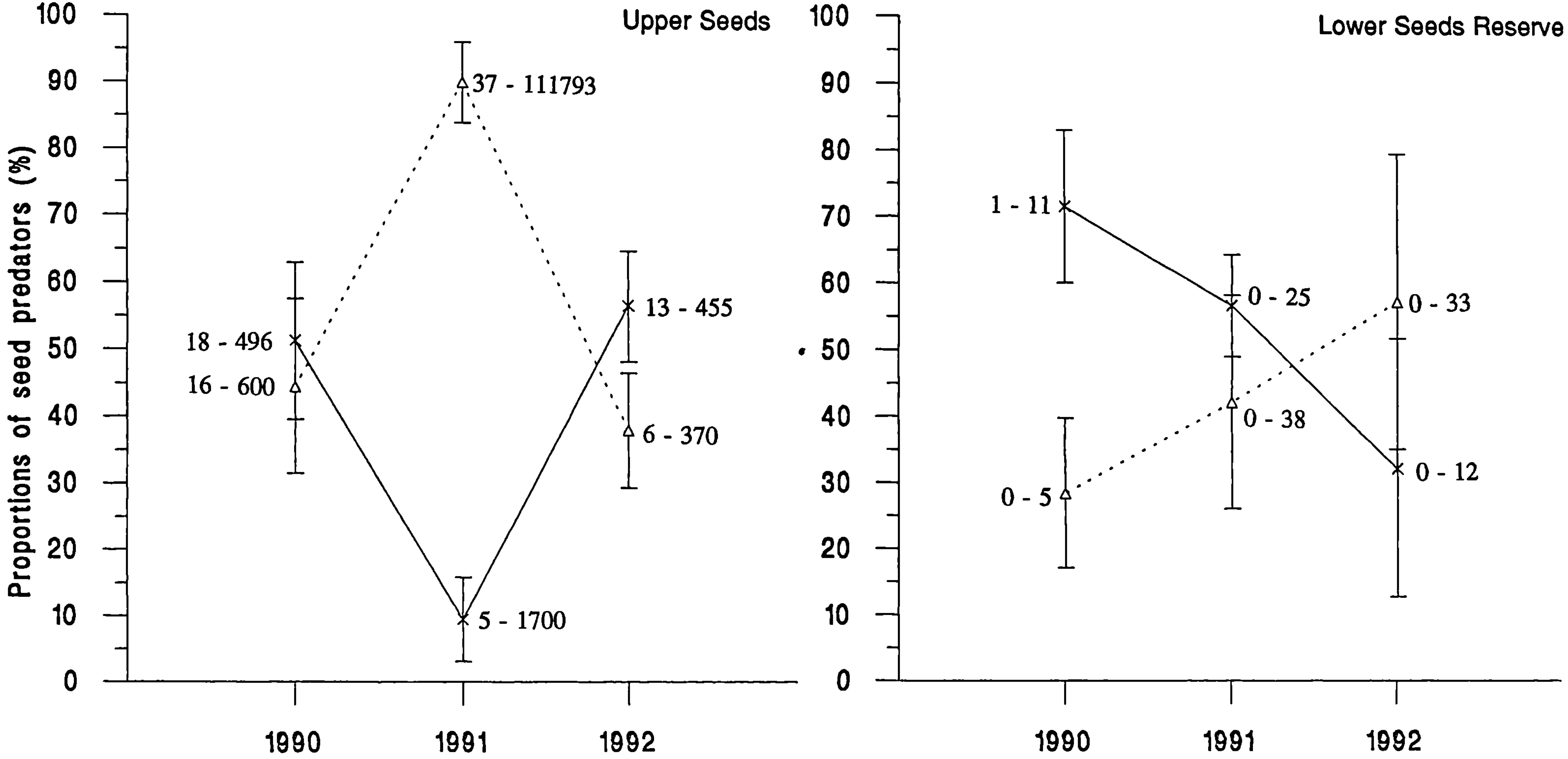

Figure 5.2: Comparison of the relative percent of weevils (——) and wasps (----) in Upper Seeds and Lower Seeds Reserve over the three years. The range of numbers of insects per plant is shown next to each data point.

3.3 Correlation of seed predation and plant traits

In Lower Seeds Reserve the proportion of pods predated is positively correlated with mean seed weight only in 1990 ($r^2 = 0.49, p < 0.02$), whilst percentage seed predation is negatively correlated with total pod production in 1992 ($r^2 = 0.38, p < 0.02$).

In Upper Seeds, in 1991, percentage seed predation is negatively correlated with percentage fruit-set ($r^2 = 0.19, p < 0.05$) and is also negatively correlated with mean pods per infructescence ($r^2 = 0.36, p < 0.03$).

Correlations with the individual seed predators are similarly inconsistent between years. In Upper Seeds in 1991, wasp numbers are negatively correlated with mean pods per infructescence ($r^2 = 0.48, p < 0.02$), which probably accounts for the negative correlation with seed predation (above). In Upper Seeds in 1992, wasp numbers are positively correlated with mean pod length ($r^2 = 0.87, p < 0.002$).

These are the only statistically significant correlations found. None of these correlations is consistent over the three years.

Within individual plants, the numbers of weevils, wasps and moths per pod were not significantly correlated with numbers of flowers per inflorescence, pods per infructescence or numbers of seeds per pod.

3.4 Flowering phenology and seed predation

The proportion of seeds predated was not correlated with timing of first or peak flowering, or duration of flowering, in Upper Seeds or Lower Seeds in any of the three years. Neither were the predation indices of the three predator species correlated with these two phenological traits. There were some correlations between synchrony and the various components of seed predation, but these were inconsistent between sites and years (Table 5.3).

Table 5.3: Correlations between components of seed predation and flowering synchrony. Proportion of seeds predated in 1990 has been calculated from proportion of pods with seed predators.

	% seeds predated		**Weevil abundance**		**Wasp abundance**	
	Upper Seeds	**Lower Seeds R.**	**Upper Seeds**	**Lower Seeds R.**	**Upper Seeds**	**Lower Seeds R.**
1990	ns	ns	ns	ns	ns	ns
1991	ns	Pearson's Correlation = 0.64; df=15; p <0.004	ns	Pearson's Correlation = 0.39; df=13; p=0.08	ns	ns
1992	Pearson's Correlation = -0.36; df=13; p=0.09	ns	Pearson's Correlation = 0.45; df=11; p=0.06	ns	ns	Pearson's Correlation = -0.51; df=8; p=0.07

In Chapter 4 it was shown that there is generally strong concordance between the phenologies of flowering and fruiting. It is known that the chalcid wasp oviposits in fertilised fruits, rather than unpollinated flowers, (Batiste, 1967) and that the weevil lays eggs on all stages of flowers, from buds to immature pods (Compton, 1983). Therefore, fruiting phenologies have been plotted in Figures 5.3, 5.4, 5.5 & 5.6. The tacit assumption of these data is that the abundance of larvae within pods from a particular part of the season reflects the activity of the adults at that time.

In some of these graphs the lines for insect activity exceed those for fruit-set because an index of seed predator effect has been used (see page 128); however, it is the shape of the graphs which is important, not the actual numbers.

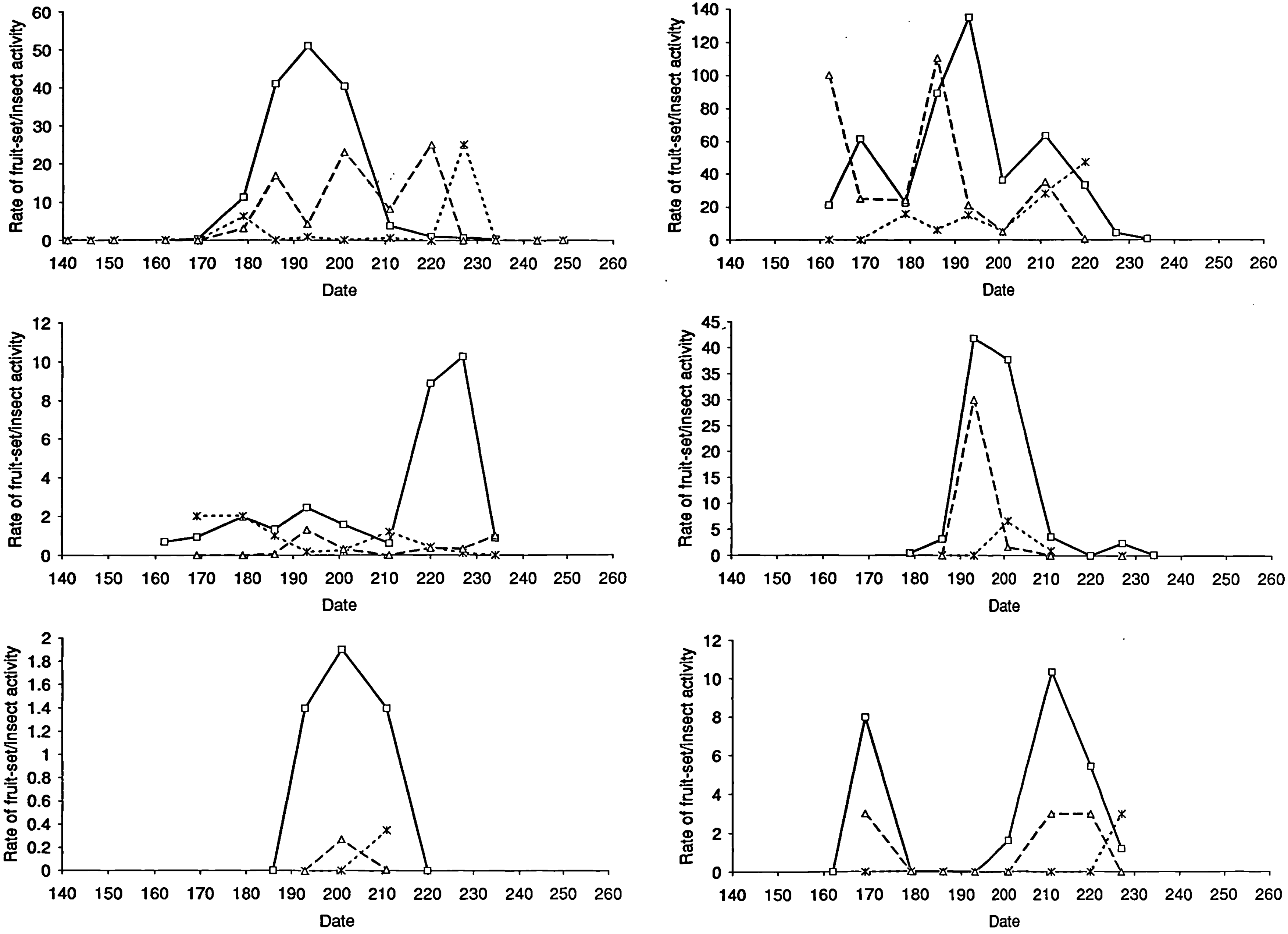

Figure 5.3: Fruiting phenology (□) and weevil (*) & wasp (Δ) activity, Upper Seeds 1991.

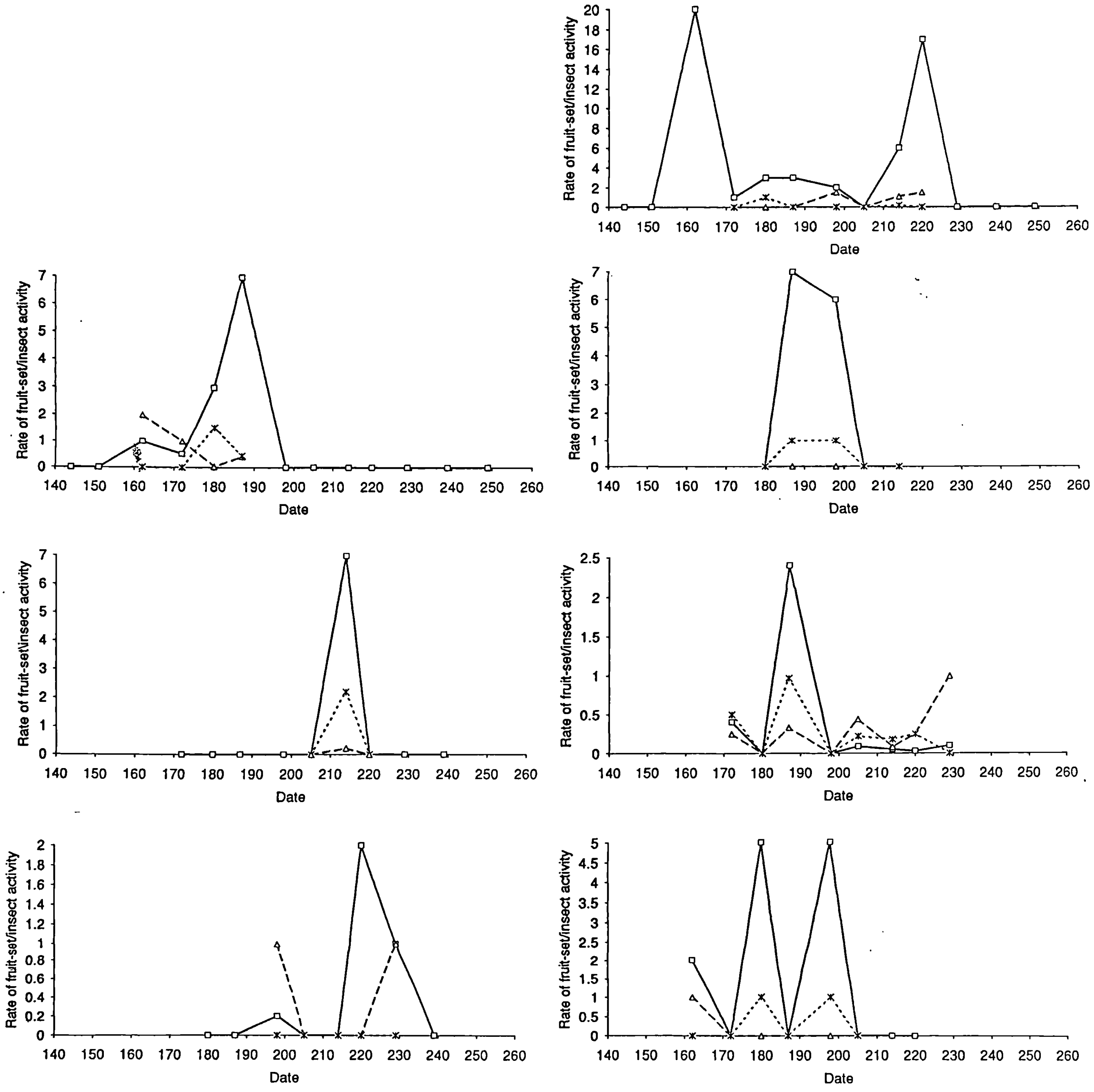

Figure 5.4: Fruiting phenology (□) and weevil (*) and wasp (Δ) activity, Lower Seeds Reserve 1991.

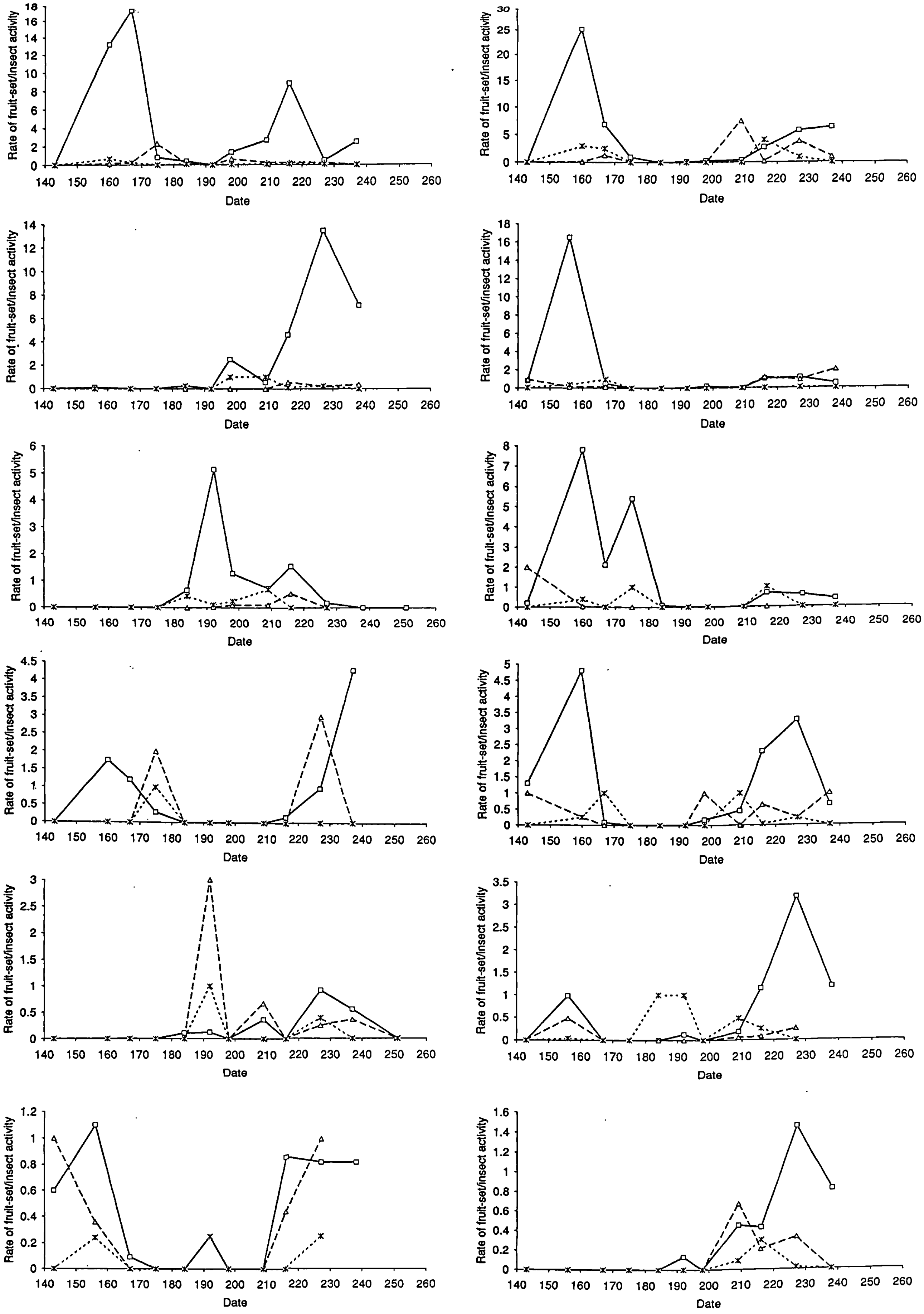

Figure 5.5: Fruiting phenology (□) and weevil (*) and wasp (△) activity, Upper Seeds 1992.

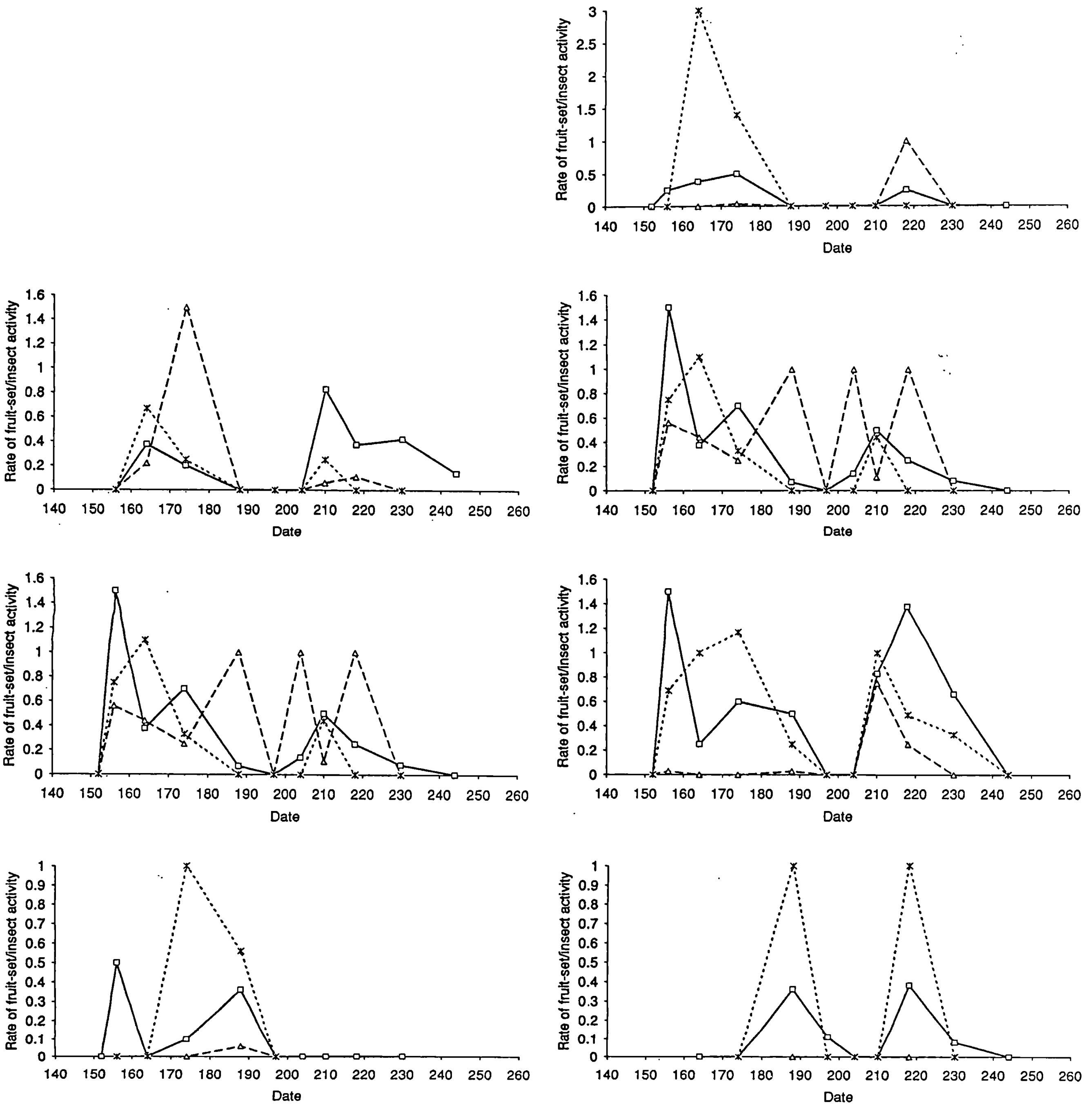

Figure 5.6: Fruiting phenology (□) and weevil (*) and wasp (△) activity, Lower Seeds 1992.

There is no indication that either the weevil or the wasp time their egg laying to periods of peak fruit-set: peak abundances of the two predators correspond to periods of peak fruiting on some plants, but not on others. There are no significant linear correlations between rate of fruit-set and weevil or wasp activity in either of the two sites in 1991 or 1992 using the combined data of all plants within sites and years. There is a strong non-linear relationship between rate of fruit-set. and weevil infestation (Figure 5.7); not surprisingly, a similar relationship is obtained for rate of flowering.The pattern is most obvious in Upper Seeds in 1991 and 1992; plants in Lower Seeds Reserve produced many fewer flowers and never had a large rate of flower production, but there is a suggestion of the same relationship on a smaller scale. At flower production rates of between 5 and 20 flowers per day weevil numbers were substantially reduced. The corresponding wasp data show no obvious patterns (Figure 5.8).

Calculating means of fruit production and insect phenologies allows one to look at site trends in the two years. This has been done using proportional fruiting data to account for differences in the magnitude of flower (and hence fruit) production between plants (Figures 5.9, 5.10, 5.11 & 5.12). Periods of low fruit-set in Lower Seeds have not been used in the calculation of mean insect activity over time as the numbers are too small to be meaningful; these times are represented by gaps in the phenologies. Despite the apparent fluctuations in the numbers of the two insects over the course of a season, a Kolmogorov-Smirnov Uniform test indicates that their numbers do not change significantly. Insect phenologies on individual plants, however, are usually significantly different from uniform, at least in Upper Seeds (**1991:** 50% of phenologies significantly different. **1992:** 80% of phenologies significantly different). In Lower Seeds Reserve, the data are too few for this analysis to be meaningful.

The combined data for all plants on wasp versus weevil abundance on each census day for the two sites in 1991 and 1992 are negatively correlated (Upper Seeds **1991:** *Kendall's tau* $= -0.37$, $p < 0.002$; Lower Seeds **1991:** *Kendall's tau* $= -0.66$, $p < 0.001$; Upper Seeds **1992:** *Kendall's tau* $= -0.63$, $p < 0.001$; Lower Seeds **1992:** *Kendall's tau* $= -0.44$, $p < 0.002$).

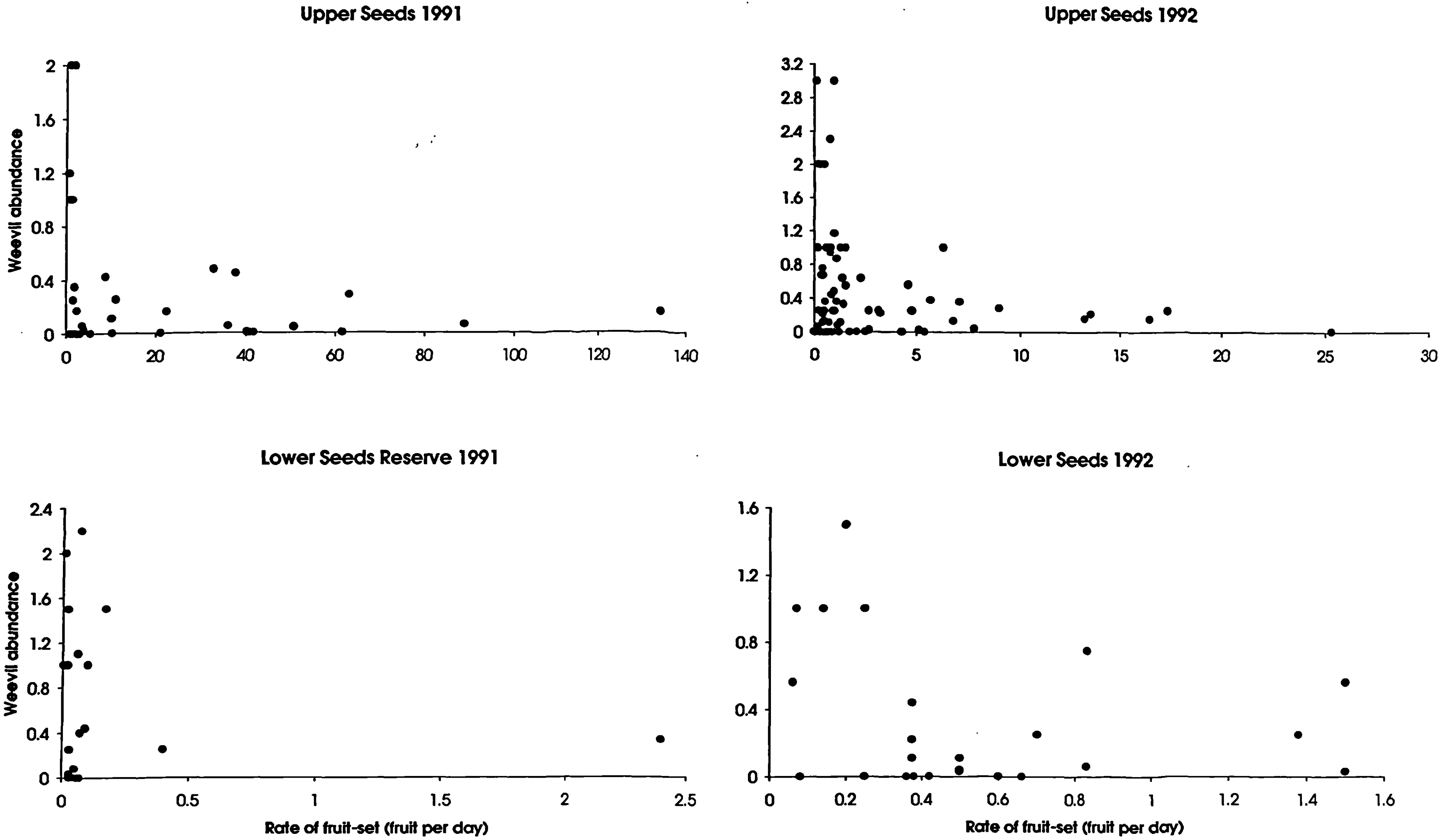

Figure 5.7: Relationship between rate of fruit production and weevil activity, Upper Seeds and Lower Seeds Reserve, 1991 and 1992.

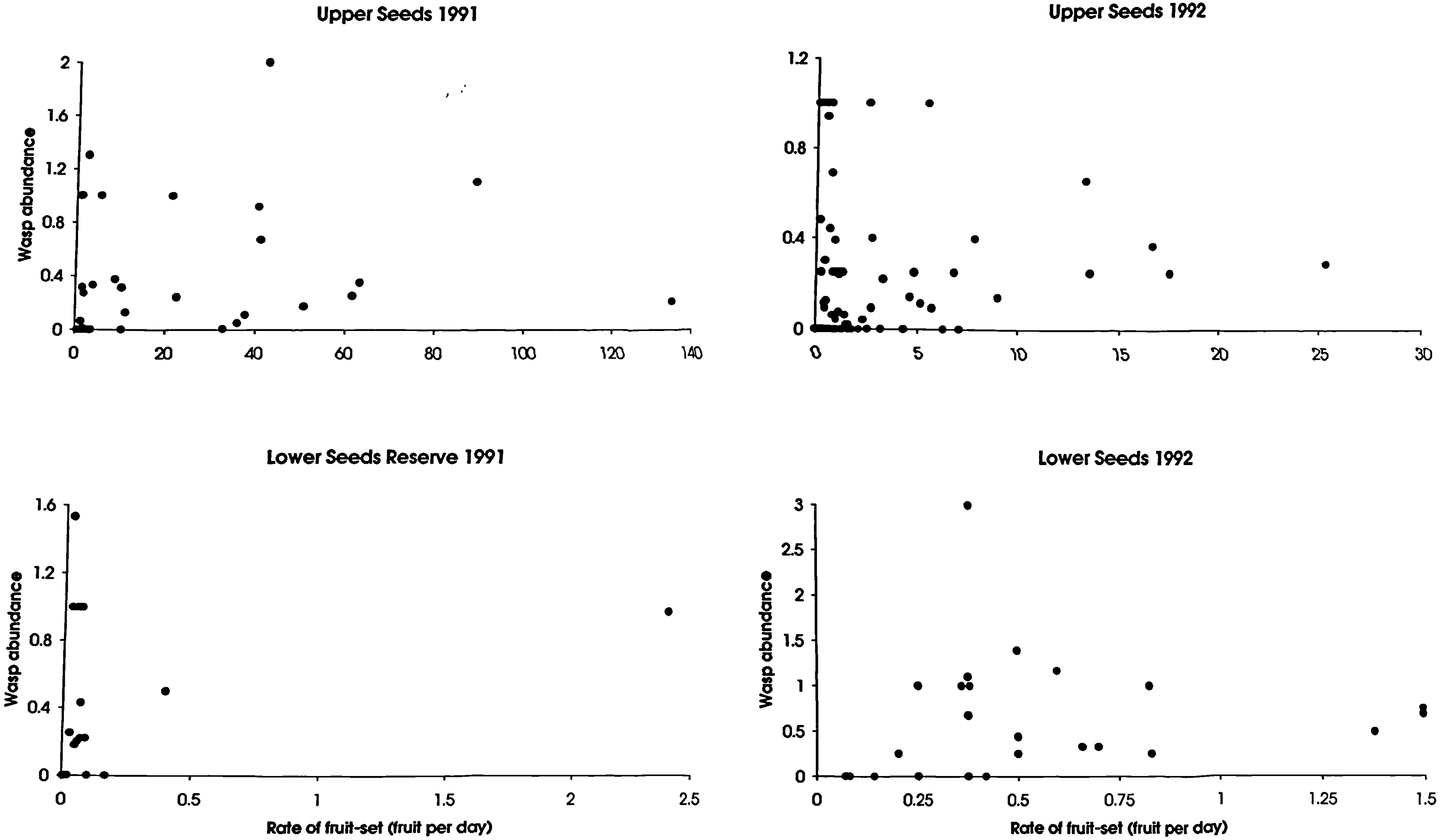

Figure 5.8: Relationship between rate of fruit production and wasp activity, Upper Seeds and Lower Seeds Reserve, 1991 and 1992.

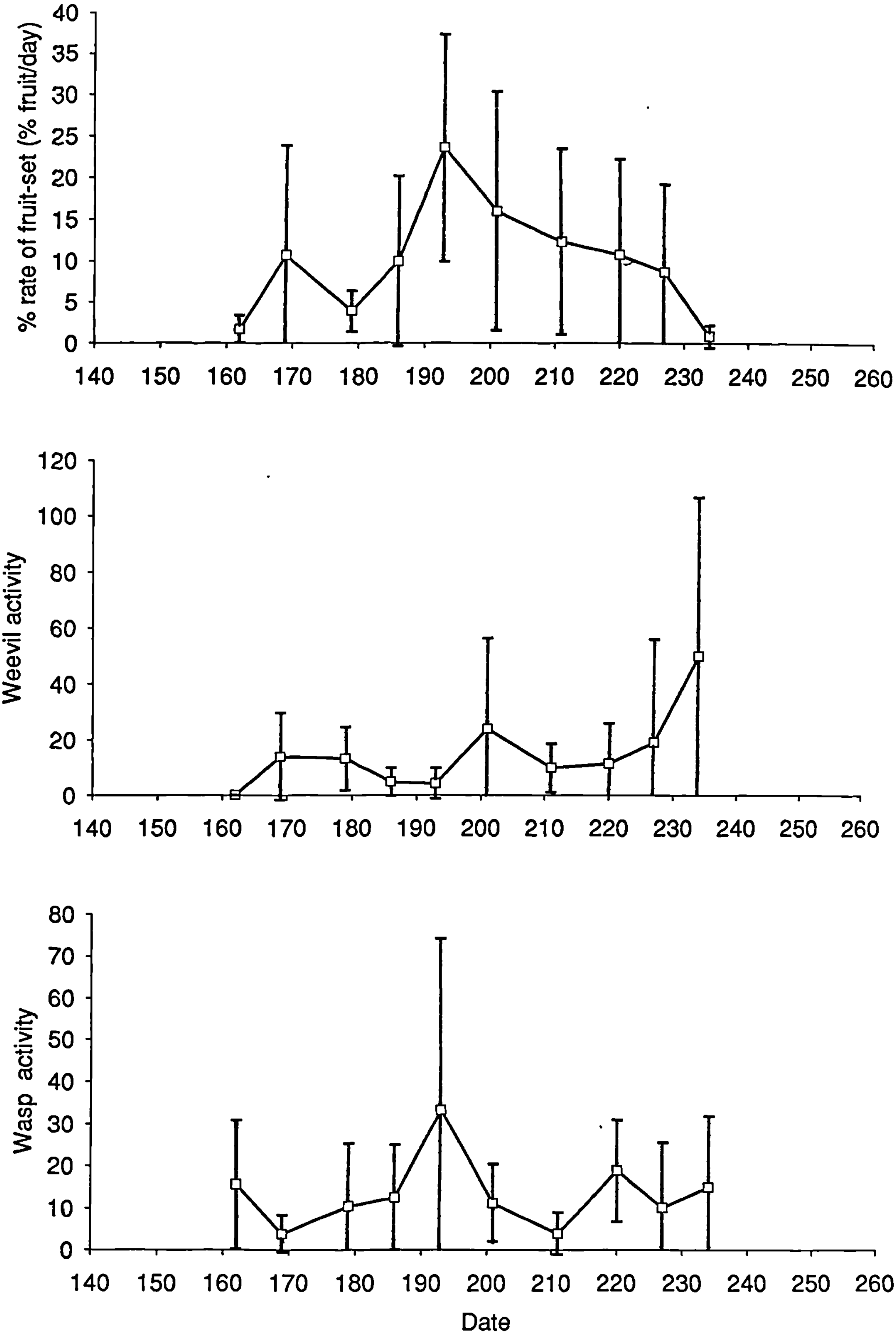

Figure 5.9: Average fruiting and weevil & wasp activity phenologies, Upper Seeds 1991.

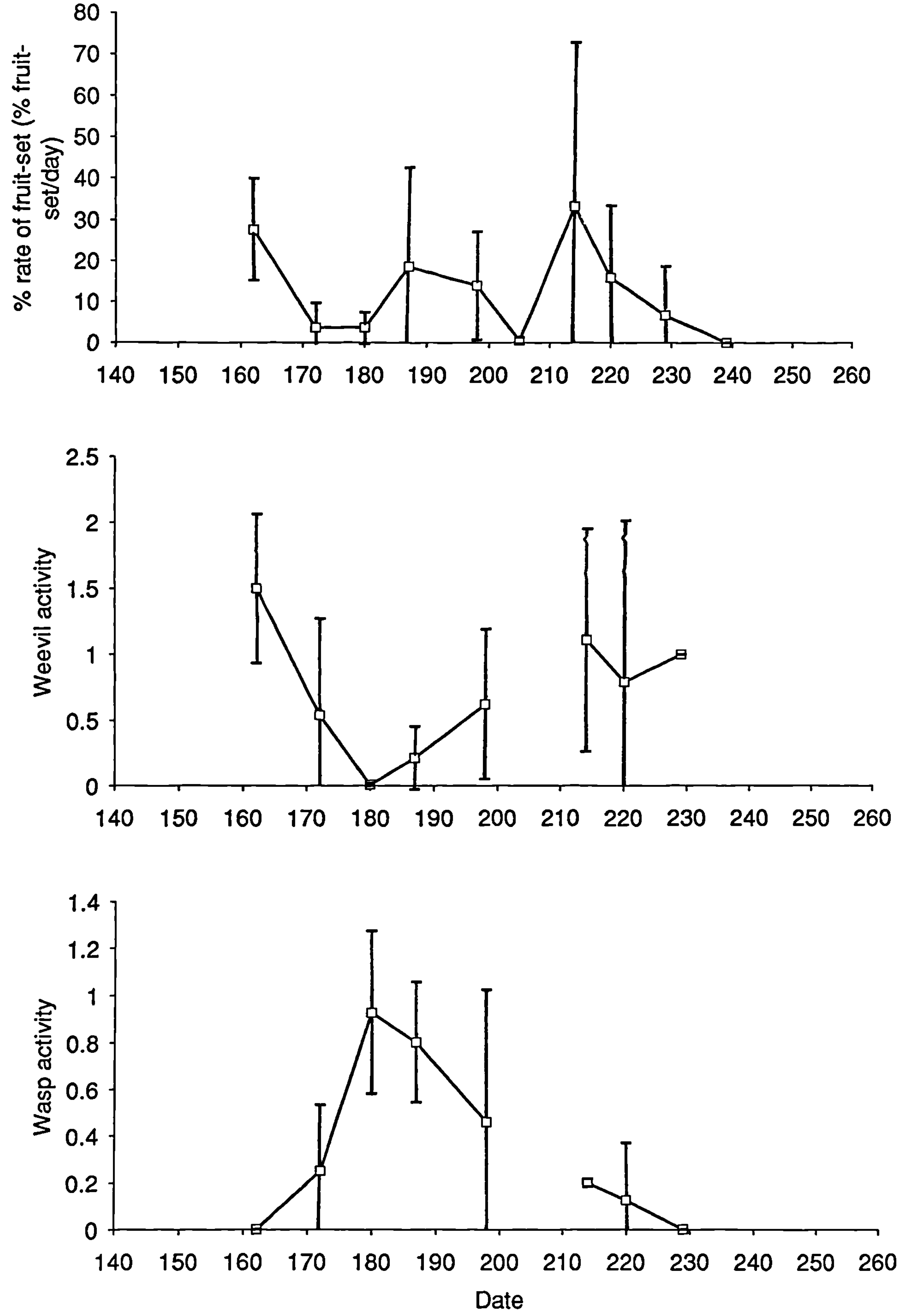

Figure 5.10: Average fruiting and weevil & wasp activity phenologies, Lower Seeds 1991.

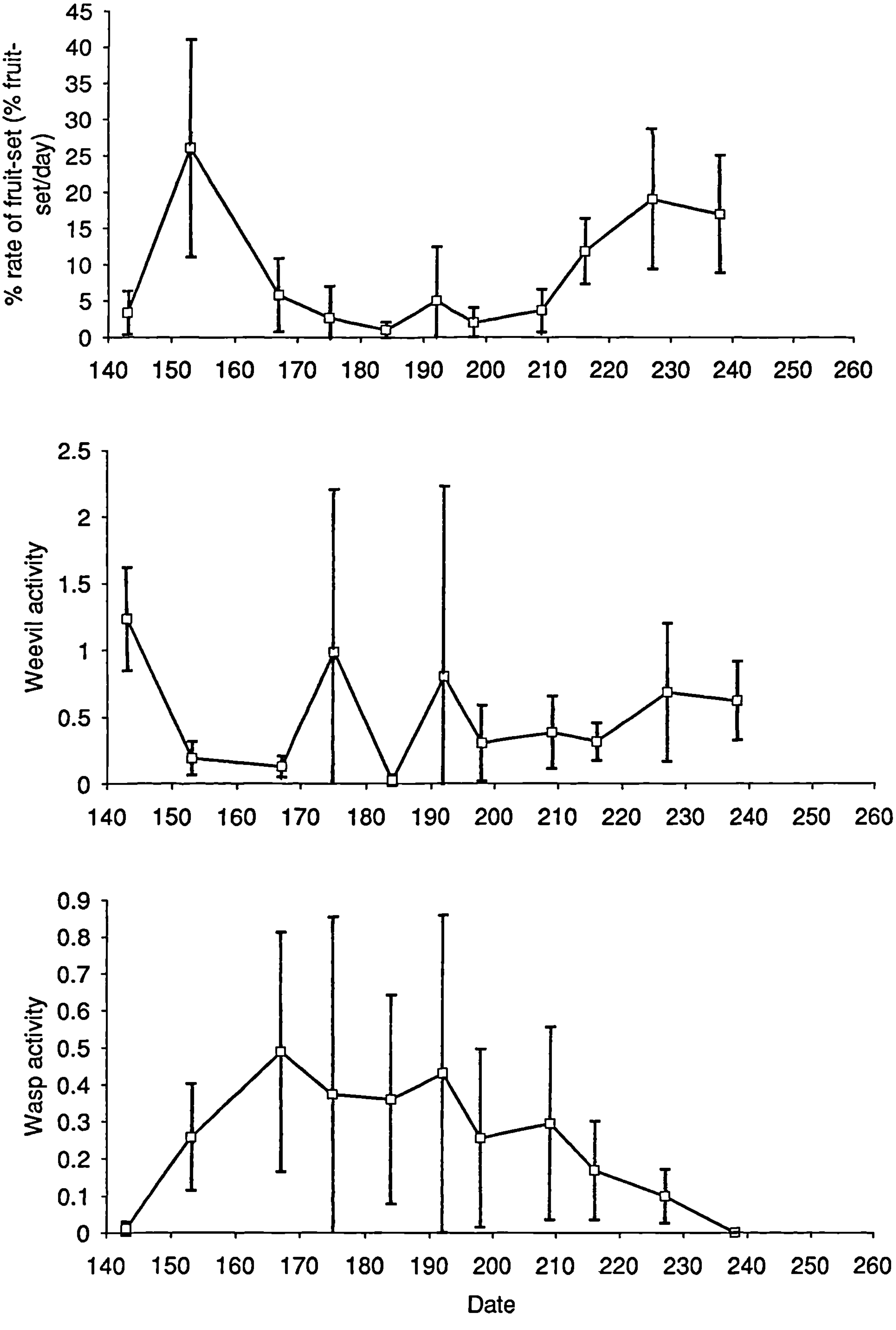

Figure 5.11: Average fruiting and weevil & wasp activity phenologies, Upper Seeds 1992.

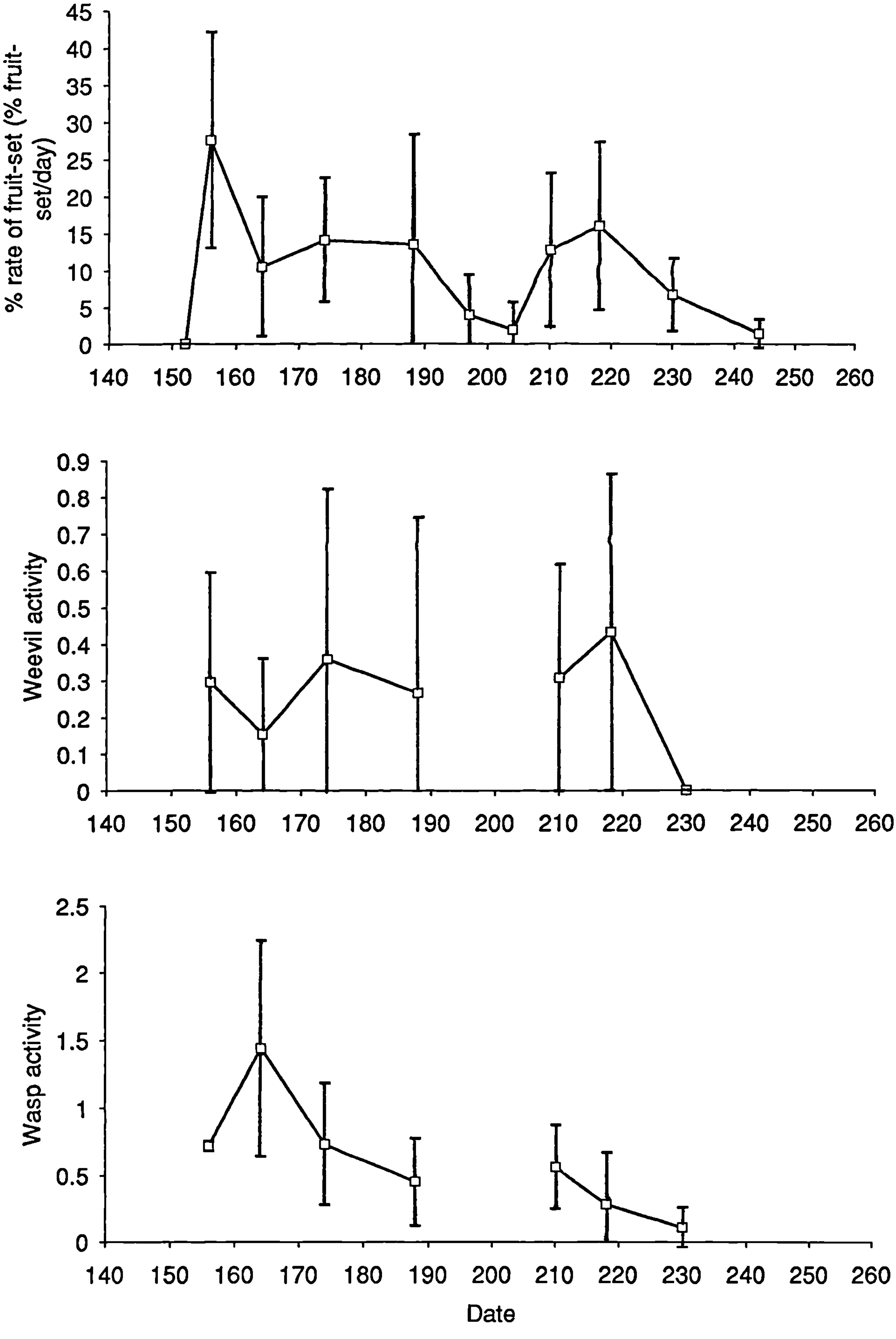

Figure 5.12: Average fruiting and weevil & wasp activity phenologies, Lower Seeds 1992.

3.5 Seed germination experiment

Speed of germination

The cumulative germination rates of the four different predation classes in the first and second runs of the experimentareshown in Figure 5.13. In both cases, the rate of germination was much faster for the damaged seeds compared with the undamaged seeds. The undamaged seeds were subsequently scarified to encourage germination.

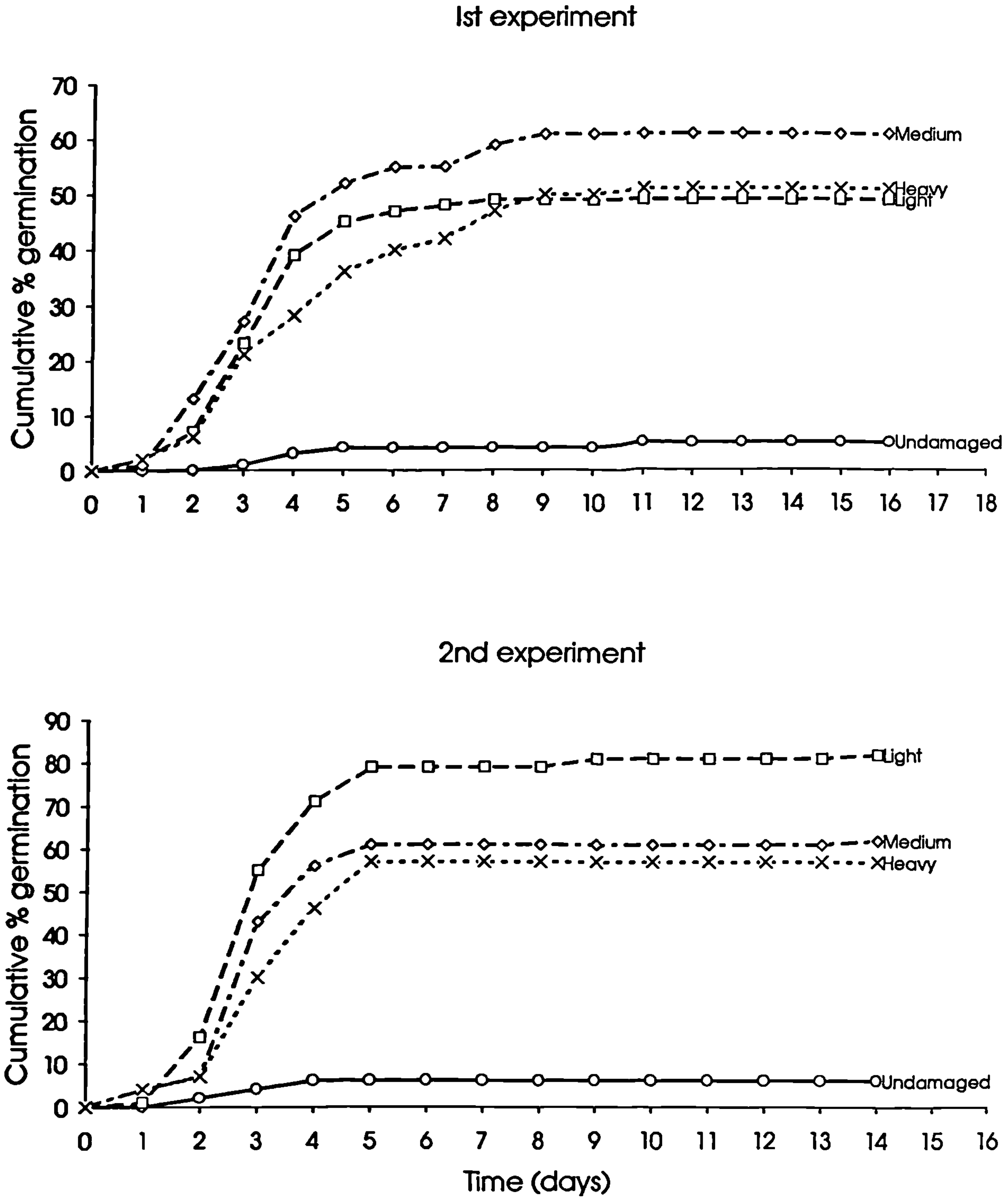

Figure 5.13: Cumulative rates of germination for the two partially-predated seed germination experiments.

Proportional germination

The percentages of the four seed classes germinating, and their fates, are shown in Table 5.4 (first experiment) and Table 5.5 (second experiment). There are differences between the two runs in terms of the much larger proportion of Light seeds germinating in the second experiment, and the fewer numbers of Medium and Heavy seedlings dying in the second experiment. The lower seedling mortality may be attributable to the seeds from the second experiment being kept at room temperature for 20 months prior to the experiment; fungal infection was the main cause of seedling death, and spore viability of many fungi is reduced under these conditions (Cochrane, 1958). The reason for the higher percentage germination of the Light predated seeds may also be linked to this.

Table 5.4: Results of the first seed germination experiment.

Level of predation	% germination	% seedlings which died
Undamaged	98.9	2.1
Light	49.0	6.1
Medium	61.0	32.8
Heavy	51.0	52.9

Table 5.5: Results of the second seed germination experiment.

Level of predation	% germination	% seedlings which died	% seedlings not establishing
Undamaged	95.6	0.0	0.0
Light	82.0	2.4	0.0
Medium	62.0	3.2	12.5
Heavy	57.0	7.0	30.0

Subsequent growth of the seedlings

The mean dry weights of the seedlings are shown in Table 5.6 together with the over-winter survivorship of the cropped seedlings; the dry weights have been compared using a separate variance t-test. The seedlings from the Heavily predated seeds are significantly smaller than those of the Light and Medium seedlings, but not of the Undamaged seedlings. In terms of over-winter survival, seedlings from the Medium and Heavy predated seeds are slightly less hardy than those from the Undamaged and Light seeds.

Table 5.6: Average dry weights (±95% confidence limits) of seedlings from the 2nd seed germination experiment and percentage over-winter survival of those seedlings. Identically numbered weights are not significantly different ($p > 0.05$).

	Mean weight (g)	±95% CI	% survivorship	n
Undamaged	$0.45^{1,2}$	0.100	83.3	6
Light	0.50^{1}	0.006	81.8	22
Medium	0.50^{1}	0.007	66.7	21
Heavy	0.40^{2}	0.005	70.0	14

A useful comparison to these results are the findings of Armstrong & Westoby (1993) who artificially defoliated seedlings; they removed 95% of the cotyledon tissue at the earliest stage of shoot development, for 22 phylogenetically independent pairs of species. They then looked at survivorship and plant dry weight after 3 weeks. I have extracted and analyzed their data for 18 species which had a seed size within the same range as *Lotus corniculatus*, that is between 1 and 20mg. The average survivorship of the clipped seedlings (± 95% Confidence Interval) was 83.8% (±12.1), whilst the mean size of the clipped seedlings (±95% C.I.) was 27.3% (±5.6) of the unclipped seedlings' size. Whilst the results for survivorship are comparable to my own, the size differences are not - the clipped seedlings in Armstrong & Westoby's study were on average much smaller than their unclipped counterparts, compared to my findings. It may be that the differences are due to the experimental seedlings having 95% of their cotyledons removed, whereas even the Heavy predated seeds rarely had this amount of damage. Three of the seedlings in my study, however, germinated with their cotyledons detached - their mean weight (± 95% C.I.) was 0.30_g(±0.1), which is 66.6% of the weight of the undamaged seedlings, and still rather greater than Armstrong & Westoby's findings. This is suggestive, but it cannot be determined with any certainty whether the seedling-size discrepancies between these two studies are the result of methodological differences or are real species differences.

When the seed pods of individual plants were assessed in 1991 and 1992, the proportions of seeds suffering the three classes of seed damage were noted. From this the proportion of the total seed output of each plant which is made up of these three classes of damage can be calculated (Table 5.7). Using these data, plus those from the first seed germination experiment (which is considered to be the more accurate of the two, bearing in mind what has been said about fungal spore viability), the proportion of an individual's seed production which is composed of viable partially predated seeds can be calculated (Table 5.7).

Table 5.7: Estimates of the proportion of viable partially eaten seeds (n=7 plants in 1991; n=10 plants in 1992). All means are ±95% confidence limits.

	Mean percentage of total seed production:			Percentage germination - seedlings died:			
	Light	Medium	Heavy	Light	Medium	Heavy	Percentage of viable, partially eaten seeds:
1991	3.7 ±1.5	3.9 ±1.8	4.7 ±1.6	46.0	41.0	24.0	4.4 ±1.8
1992	4.6 ±2.5	5.9 ±6.3	2.4 ±1.9				5.1 ±4.3

4. Discussion

4.1 Variation in seed predation

The proportion of seeds lost to seed predators was variable between plants. This variation was not consistent between years (that is to say, plants which were heavily predated in 1991 were not more likely to be heavily predated in 1992); nor is it definitively linked to any of the plant traits examined (*i.e.* total flower and pod production, mean inflorescence and infructescence size, mean numbers of seeds per pod, and average seed size). There is therefore no evidence from this study that natural selection is acting in any consistent way on these plant traits through seed predation. For example, the negative correlation between infructescence size and wasp predation, if consistent over time, could result in selection for plants with larger infructesences. But this relationship was only found in 1991, which suggests that it is either inconsistent over time, or may be a spurious correlation. Franson & Willson (1983) also found that in *Asclepias syriaca* (Asclepiadaceae) large follicle "clusters" (*i.e.* infructescences) were less heavily predated than smaller ones. Nevertheless large infructescences were not produced more frequently than expected in the population. This could be because infructescence size is not a heritable trait (as it is in *L. corniculatus*; Jones & Turkington, 1983) or some other factor may be affecting the fitness of those individuals.

Seed predation has been implicated as a selective agent for some plant characteristics. Herrera (1984) looked at fruit seediness in two populations of *Berberis hispanica* (Berberidaceae). A positive correlation between number of seeds per fruit and degree of seed predation by a dipteran larva existed in the first population. In the second population, where the dipteran was much rarer, mean number of seeds per fruit was significantly higher than in the first. Zimmerman (1980) also found greater predation in seedier fruits of *Polemonium foliosissimum* (Polemoniaceae). This study was only done over two years and no conclusions can be drawn about the likelihood of the relationship affecting numbers of seeds per fruit, particularly because, as I have shown, these relationships can change over time. Most studies of seed predation are done over one or two years, rarely three or four, and Herrera's work is the only one I have found which compares contrasting populations of the same species. Looking at a plant/predator system for a longer period of time may not necessarily clarify the role of seed predators as selective agents. The approach of Herrera, on the other hand, is one which should be more widely attempted.

In his study of the insects associated with *Lotus corniculatus*, Compton (1983) found a positive correlation between the numbers of seeds per pod and the numbers of chalcid wasps per pod, leading him to conclude that the wasp preferentially oviposits in pods with more seeds. Compton assessed this only in 1977, and my study has found no evidence to support his hypothesis.

The proportions of the three seed predators infesting individual plants is also variable, and numbers of weevils and wasps fluctuate over the three years, though moth numbers are consistently low. The weevil was the most abundant of the three seed predators found at all sites in Compton's (1983) study, though the moth was more abundant in his sites than in mine; its presence was recorded in up to 25% of pods. This difference in our findings may be because of the large amount of *Trifolium* spp. at Wytham, which is the main food plant of this microlepidopteran (Compton, 1983).

The lack of between-year concordance in the numbers of each of the three insects on individual plants may be testament to the vagility of the adults of these species, though this is not the only possible explanation. Consistently high infestation rates on the same individuals have been found in some insect/plant systems, even when the animal concerned is highly mobile, and experimental work has ascribed this to assortative interactions between the plant and the insect, caused by genetic sub-structuring of the population (Weiss & Campbell, 1992). This is further evidence that factors other than the specific plant traits which I examined are important in determining the level of seed predation an individual plant will suffer in any one year.

Plant size is known to be an important shaper of seed predator behaviour and subsequent seed mortality in some species. For example, Hainsworth *et al.* (1984) examined the effect of plant size on pollination and seed predation in *Ipomopsis* *aggregata* (Polemoniaceae). Larger plants had greater seed predation, but this was more than compensated for by increased rates of pollination, and therefore fruit-set. Once again, this was a one year study. The data presented here on *Lotus corniculatus* indicate that, in this species at least, plant size is not an important determinant of seed predation.

4.2 The interaction of seed predation and flowering phenology

My results show that there is unlikely to be selection acting through seed predation on flowering time in *Lotus corniculatus* at Wytham. A number of studies have implicated seed predation in the moulding of species' flowering patterns. Augspurger (1981) found that individuals of *Hybanthus prunifolius* (Violaceae) which flowered out of synchrony with the rest of the population were preferentially attacked by microlepidopteran seed predators. English-Loeb & Karban (1992), on the other hand, found that asynchronous plants of *Erigeron glaucus* (Compositae) avoided seed predation by tephritid flies. Zimmerman & Gross (1984) looked at seed predation of *Polemonium foliosissimum* (Polemoniaceae) by dipteran larvae, and showed that the degree of predation was negatively correlated with timing of flowering, such that later flowering plants fared better. Negatively correlated flowering and seed predation, in some years, was also the finding of Evans *et al.* (1989), working with *Baptisia australis* (Leguminosae), though this is likely to have been offset by greater herbivory of flowers and reproductive tissue by beetles later in the season. Evans and his co-workers believed variability of seed and flower predation between years to be the factors maintaining flowering asynchrony. Green & Palmbald (1975) found that later flowering plants of *Astragalus cibarius* (Leguminosae) were more heavily predated, which they took as evidence of selection for earlier flowering, resulting in phenological differences between *A. cibarius* and its less-heavily predated congeneric *A. utahensis*.

All of these studies inferred a genetic basis to flowering time, and therefore the potential for natural selection to act. Only two of them had more than one year's worth of data. Evans *et al.*'s (1989) three year study found that the pattern of predation was different from year to year; that of Zimmerman & Gross (1984), which was undertaken over four years, found that in three out of the four years there was a significant, though very weak, negative correlation between seed predation and flowering time. A third investigation was also longer term: Pettersson's (1992) three year study of seed predation in *Silene vulgaris* var. *petraea* documented heavier seed predation in early flowering plants, compared to late flowering ones, in two out of the three years of the study. However, Pettersson's conclusion was that any selection through seed predation was too inconsistent to mould flowering phenology in this species.

It comes as no surprise to find that the exact interaction between plants and their seed predators is strongly species-specific. However, it also seems as if these interactions are variable between-years (and probably between-sites), and one should be cautious about inferring selection from a limited study. Natural selection, after all, acts on lifetime fitness. It is not impossible for natural selection to be inconsistent between years and yet still result in differential fitness; it is simply less likely.

There must be some degree of concordance between seed predator activity and flowering or fruiting phenology of the host species, but the specific timing of events has rarely been established for these plant/animal interactions, despite the large volume of seed predation literature. In *Lotus corniculatus*, the two main seed predators do not appear to be keeping pace with flower or fruit production, and in the case of the weevil, insect numbers can only keep pace with numbers of flowers up to a rather modest rate. The reasons for the difference between the two insects is not known. The few published studies record little or no concordance between flowering and seed predator phenologies: for example, there was no relationship between population phenology of *Trollius europaeus* (Ranunculaceae) and its four species of pollinating/seed predating mutualist *Chiastocheta* (Diptera: Anthomyiidae) flies (Pellmyr, 1992). Similarly, the data given by Grieg (1993) show no concordance between the population flowering phenologies of three species of *Piper* (Piperaceae) and the combined predatory effects of a number of hemipteran and coleopteran seed eaters. In Grieg's data there seems to be a pattern of high seed predation at times of low flower production, and vice versa.

Should we expect a strong correlation between the phenologies of a plant species and its seed predators? If the relationship is a close one (*i.e.* species-specific, as in the case of *Lotus corniculatus* with *Apion loti* and *Eurytoma platyptera*) then the intuitive answer is: "Yes" - there should be strong selection for those insect individuals whose activity is timed to periods of peak flowering in their hosts. However, the flowering phenologies of individuals of *Lotus corniculatus* are variable between years (see Chapter 4); this shifting of flowering time gives no opportunity for selection to act on the life histories of the seed predators, so the rather loose concordance between host and predator phenologies is not surprising.

4.3 Interactions between the weevil and the wasp.

It appears from the phenological graphs and the correlations that some kind of interaction is going on between the weevil and the wasp which means that numbers of one are high when numbers of the other are low on individual plants. There are three pieces of evidence to support this.

1. On individual plants, times of high weevil abundance tend to coincide with periods of low wasp abundance (Figures 5.3, 5.4 5.5 & 5.6).

2. There are negative correlations between weevil and wasp abundance, for all plants combined, in Upper Seeds and Lower Seeds Reserve in 1991 and 1992 (page 142).

3. Years of high weevil numbers generally correspond to years of low wasp number (Figure 5.2).

From what is already known about the ecology of these two species, the following hypotheses may account for this interaction:

1. The weevil may be directly affecting wasp numbers by its feeding. The wasp spends the portion of its life cycle from egg to pupa within a single seed, whilst the weevil spends its early life cycle within an entire pod. Compton (1983) has already established that wasp larvae whose seeds are damaged usually die, probably from dehydration. On the basis of their behaviour and life histories, he surmised

that there was a hierarchy of interaction between the moth, weevil and wasp. The moth is likely to affect numbers of the other two species because it is the most mobile and can move between pods, eating everything in those pods, including eggs and young larvae. The weevil is intermediate because, although it can damage wasp-containing seeds, it remains within one pod. The wasp is least likely to affect the other two species because it stays within a single seed until pupation. In the absence of large numbers of moths, it may be that it is the weevil which is inadvertently killing wasp larvae by its feeding.

2. The wasp could be avoiding those pods which contain weevil larvae. Compton (1983) thought that he had some evidence for this, and the hypothesis fits in with what is known about the oviposition behaviour of the wasp. Batiste (1967) showed that it preferentially oviposited on pods 10 to 12 days post-pollination. The weevil, on the other hand, will lay its eggs at any stage, but often even before the flower is fully open. This gives the wasp the opportunity to avoid some weevil larvae, at least, if it can detect their presence. Ovipositing females of a number of insect species, including weevils, are known to mark their egg-laying sites with conspecific-deterring pheromones (Prokopy *et al.*, 1984). This could be one way for the wasp to avoid laying eggs in the same pods as the weevil, if the wasp can detect any scent that the weevil was leaving behind.

These two hypotheses can, in fact, be validly combined. Experimental manipulations would be necessary to determine exactly the nature of this interaction.

4.4 The significance of partial seed predation

The seed germination experiments have shown that a proportion of the partially eaten seeds dispersed by a plant is still viable, and can germinate and produce seedlings which are almost as vigorous and hardy as seedlings from unpredated seeds[2]. These seeds make up a small, but not insignificant, fraction of a plant's

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[2] Jones *et al.* (1986) also tried to germinate predator damaged seeds, apparently without success. However, they assumed that the fraction of seeds which did not germinate included all of the damaged seeds, but conceded that "it was not possible to examine all 31,704 seeds individually for evidence of damage".

total seed output. Thus, the figures for seeds predated given in this study (see Table 5.7) have been over-estimated by about 5%. Over twenty years ago, Janzen (1971) pointed out that assessing pre-dispersal seed predation was rather more complex than simply observing damage to seeds. Much more recently, the unstraightforward nature of this area of plant reproductive ecology has been shown in some studies; for example, Molau *et al.* (1989) found that undamaged seeds from predated fruits of *Bartsia alpina* had a lower germination rate than seeds from unpredated fruits. Andersen (1988) has shown that traditional (*i.e.* observational) assessments of predation may be as much as an order of magnitude too low, because predation may reduce the total number of seeds produced by a pod and/or reduce the remaining seed viability. Differences in seed viability were not looked for in this study, so it is not possible to discount this effect in *Lotus corniculatus*, but as I have shown in Chapter 3, seed size is unaffected by seed predation. There is also no difference in mean pod length of predated versus unpredated pods, indicating that seed predation is not affecting the total numbers of seeds per pod. It is likely that the effects noted by Molau *et al.* (1989) and Andersen (1988) are strongly dependent upon the species under consideration.

In the vast majority of seed predation studies, no account is taken as to the viability or otherwise of damaged seeds; those seeds which appear to have been partially eaten are simply included within the figure of "seeds predated". During a literature search of pre-dispersal seed predation studies, I encountered only five papers in which an assessment of the viability of "predated" seeds had been made. Green and Palmbald (1975) used the tetrazolium test to show that none of the damaged seeds that they collected from *Astragalus cibarius* and *A. utahensis* were viable. The tetrazolium test is a standard method of determining seed viability in the agricultural and horticultural sciences (Moore, 1973), but has not been widely used by ecologists. El Atta (1993) recorded zero germination from seeds of *Acacia nilotica* (Leguminosae) which had been infested with larvae of the bruchid beetle *Caryedon serratus*. No measure of the degree of damage was made but, as the beetles spend their entire larval stage within one seed (*c.f.* the wasp *Eurytoma platyptera* in my study), and a number of larvae feed in each seed, it is likely that the entire contents of a seed are destroyed. The other three studies (Ellison & Thompson, 1987; Ernst *et al.*, 1989; and Robertson *et al.*, 1990) recorded varying degrees of viability in partially-predated seeds. Ellison & Thompson (1987) reported that 6% of the partially-predated seeds of *Lomatium grayi* (Umbelliferae) were still viable. Ernst *et al.* (1989) in a study of *Acacia tortilis* (Leguminosae) found that damaged seeds imbibed water and germinated faster than undamaged seeds, but the percentage germination of these seeds was very low (0 - 3%). This study was an attempt to test a proposal by Halevy (1974, cited in Ernst *et al.*, 1989) that insect damage, and subsequent ingestion by gazelles, would improve germination rates by breaking the hard-coat dormancy. Ernst and his co-workers seem to have disproved that idea.

In a study of the impact of seed predation on seed viability and seedling growth in Australian mangrove species, Robertson *et al.* (1990) found that a proportion of the damaged seeds from each species could remain viable; for example, there was germination of 22% of the damaged seeds of *Xylocarpus granatum* (Meliaceae) but seed damage resulted in a reduction of seedling vigour in some (but not all) of the species studied. They hypothesised that, because of the intense seedling competition found beneath the mangrove canopy, seedlings resulting from damaged seeds would be unlikely to survive, but did not attempt to test this hypothesis.

These three studies confirm that the results presented here for *Lotus corniculatus* are not unique, and hold true across habitats and phylogenetic relationships. I would hypothesise that the negative effects on seedling growth suggested by Robertson *et al.* (1990) would not be quite so important in the calcareous grassland being studied here, as seedlings of *L. corniculatus* are infrequently encountered; density-dependent regulation of seedling establishment is not likely to be an important factor in determining which seedlings are recruited into the population at Wytham. Thus the slight loss of seedling vigour found in the heavy predated seeds, and the rather reduced hardiness, may be immaterial in the context of competition with conspecific seedlings, though not necessarily in competition with other species.

Why is it that seeds (of some species at least) can survive predator damage, and produce seedlings as vigorous as those from undamaged seeds? McKersie *et al.* (1981) found that, in *Lotus corniculatus*, seed size was not correlated with viability, but was linked to seedling length after seven days and to subsequent establishment in the field. They suggested that perhaps this was due to greater stored food reserves in the larger seeds, but referred to other studies in which biochemical and developmental differences had been pin-pointed. If seed damage can be viewed as a reduction in seed size, and thus in stored food reserves, it appears that *L. corniculatus* seeds can, down to a lower limit, produce viable, vigorous seedlings, of more or less the same size from a range of seed sizes. This may explain the seed size variation found in this species (see Chapter 3); if individuals with variable seeds are not being selected against by a loss of vigour in the seedlings resulting from smaller seeds, the phenomenon will persist.

The greater rate of germination of the part-predated seeds was due to the hard seed coat being breached. Approximately 94% of the viable, unpredated seeds from Wytham are hard-coated, which is comparable to the 97.8% quoted by Jones & Turkington (1983). These seeds have a long viability and can form a persistent seed bank (Jones & Turkington, 1983) and it may take several years for them to germinate. The "soft" seeds will mostly germinate in the autumn that they are shed. So will the part-predated seeds, which means that the numbers of seedlings emerging from that year's seeds would be more than doubled if one takes into account the damaged seeds. The implications of this for the population dynamics of the species are not known, though at the very least it would result in inaccuracies in demographic data if one were measuring seed rain and relating it to seedling emergence.

Chapter 6: Conclusions

1. The reproductive output of *Lotus corniculatus*

2. The flowering phenology of *Lotus corniculatus*

3. Seed predation and the evolution of plant traits

4. Final comments

1. The reproductive output of *Lotus corniculatus*

I do not believe that the hypothesis concerning variation in reproductive output as a function of plant size was adequately tested, as it seems likely that plant size *per se* is not a good indicator of resource status. Part of the problem is in how to define "resource status": with regard to this hypothesis, a plant with a high resource status would be one which had an excess of nutrients available for expenditure on more, larger seeds and/or fruits. But such an excess of nutrients might serve a plant better if they were diverted into the iteration of new modules, if it was in a situation in which canopy competitiveness was favoured; or flowers, if floral display were important; or roots, if root competition was fierce. All of these scenarios depend upon the kind of habitat the plant is growing in. But this would not dictate the pattern of resource use; that would depend upon the evolutionary history of the taxon, and the kinds of habitats its ancestors grew in, which may have been different from the habitats in which it is presently found. If plant communities are really impermanent features in changing landscapes, as some would argue, is it likely that plants have ever evolved optimal resource use adaptations? In such a circumstance, might not a flexible strategy be the one which is most optimal? After all, we expect flexibility in many other plant traits, so why not plastic resource allocation? If this model were correct, it would be the habitat and growing circumstances of each individual which dictates how resources are apportioned, not simply the amounts of those resources.

These ideas are far from new; over 25 years ago Harper (1967) posed two questions: "Is the proportion of a plant's output that is devoted to reproduction higher in colonising species than in those of mature habitats?" and "Is the proportion of a plant's output that is devoted to reproduction fixed or plastic? Is it changed by inter- or intraspecific competition?". Since then, evidence has accumulated that some species do have a flexible response in different habitats (see discussion by Weiner, 1988) but this is almost invariably considered as allocation to reproduction *versus* vegetative growth. Some work has been done on population differences in trade-offs between the components of reproduction, for example De Ridder's (1990) study of *Drosera intermedia* (Droseraceae), but no one seems to have considered variation in trade-off relationships between individuals in the same habitat. This is an area in which further work is needed.

I also feel that the concept of "reproductive costs" is one which may have less significance for plants than animals, and that it is a concept which has been accepted too uncritically by plant ecologists. It is difficult to make sense of the fact that *Lotus corniculatus* regulates flower production at the level of the inflorescence, rather than not aborting "excess" flower primordia, in the light of their (presumed) relative reproductive costs. The situation appears wasteful from the plant's point of view, unless some other factor is selecting for small inflorescence size.

2. The flowering phenology of *Lotus corniculatus*

There appears to be no adaptive significance to the pattern of flower production in this species; I have found no evidence that the observed asynchrony reduces either competition for pollinators or the effects of seed predators. If it did, I would expect that those individuals with more synchronous flowering patterns would have lower fruit-set and higher seed predation; this is not the case. If flowering asynchrony is adaptive, this means that either the asynchrony is being maintained by other selective factors (for example, increased paternal fitness) or the trait evolved so long ago that any synchronous variants have been eliminated from the gene pool. However, the lack of correlation between the genetically determined first flowering date and flowering synchrony implies that, even if there were selection acting on the latter, it would be phenotypic selection (*sensu* Endler, 1986) only, and therefore not adaptive. I have argued elsewhere about the non-adaptive nature of many species' flowering phenologies (Ollerton & Lack, 1992 - see copy in Appendix 1) and this study has provided no data which contradict this stance. Having said that, I do not believe that lowland Britain is the best place to test ideas concerning the adaptiveness or otherwise of flowering patterns. The landscape of Britain is testament to a history of intensive agriculture and industry; there are few, if any, fragments of the kinds of habitats which existed prior to the alterations of human activity. What effect has this large-scale disturbance had on the ecology of the plants which now grow here? Recent work by Bush (1993) has documented vegetational and invertebrate changes at a British chalk grassland site for the last 11 400 years. His findings show that, though species may persist in an area over several thousands of years, the community context in which they are found can change. Selective agents of traits such as flowering time may not have the opportunity to act, as the correspondence of species and selection changes. It is well documented that flowering time can respond rapidly to selection (Ollerton & Lack, 1992), but I do not believe that this opportunity has been afforded many species in lowland Britain in the last 10 000 years or so. If there has been selection for flowering synchrony in any British species (which may not have been the case - see Ollerton & Lack, 1992) habitats have been too transitory to allow this selection to act; genotypes with earlier or later flowering times have persisted because there has not been selection against them. It would be instructive, therefore, to contrast comparable habitats from highly settled and wilderness areas, to see if variable flowering times of species correlate with the former but not the latter.

3. Seed predation and the evolution of plant traits

Whilst my study does not disprove the possibility that seed predators have been important selective agents in shaping the morphology (and flowering phenology) of *Lotus corniculatus*, I do have evidence that it is unlikely, at least for those traits which I studied. This evidence consists of a lack of between-year consistency in the degree of seed predation suffered by individuals possessing particular traits. Though this is not a study of natural selection *per se*, quantifying seed loss in this way is a first step and at least gives an indication of fruitful avenues that might yield examples of natural selection acting on plants. The absence of selection, if that is what I have identified, means either that seed predation has acted in the past to remove certain traits from the gene pool (for example, large mean inflorescence size) or those traits which I examined are not important ones as far as the behaviour of seed predators is concerned.

5. Final comments

When this work is considered, the overriding conclusion has to be that the reproductive ecology of *Lotus corniculatus* is variable; the features I have looked at are variable between individuals, between sites and from year to year. This has implications for the way in which we study the ecology and evolution of a species. If I had looked at *Lotus corniculatus* only in 1991, at the Lower Seeds Reserve site, these would have been some of my conclusions about its reproductive ecology.

1. There is no relationship between the size of a plant and the number of flowers it produces (Figure 3.7).

2. Neither sunshine nor rainfall can explain flowering patterns (page 94).

3. First flowering date is highly correlated with flowering synchrony, implying that there is a genetic basis to synchrony (Table 4.8).

4. Plants which flower more synchronously with the population are preferentially attacked by weevils and suffer greater seed predation, therefore selection is probably acting against those plants which flower synchronously, implying that asynchrony is adaptive (Table 5.3).

None of these conclusions is true: all of the evidence underlying them varies between sites and/or years. Variation in the ecology of a species on this kind of scale is rarely documented in the literature: does this mean that *Lotus corniculatus* is unusual, or are other studies missing the variation, either because they were carried out over too short a time period or in too few sites? Three years and two sites is not sufficient for a study of any species, and I would be circumspect about forming too many conclusions about what is and is not important regarding the ecology of flowering and fruiting in *Lotus corniculatus*. It is only recently, with the advent of modern molecular methods for probing the genetics of organisms, that the importance of genotypic variation between individuals of the same species has been recognised. There is reason to believe that these differences could be of fundamental importance in determining community structure at all levels (see chapters in Hunter *et al.*, 1992). If we overlay onto this genotypic variation, ecological variation of the kind I have documented (some of which may itself be caused by these genetic differences) we are left with a stunningly complex scenario of potential interactions, which we are only just beginning to come to terms with.

The positive, linear relationship that I have described between size and reproductive output has important consequences for natural selection within a population. Plant size, and hence flower production, is a phenotypically plastic trait which is influenced by an individual's age and/or growing conditions. Though there may be a small genetic component to flower production in some species, as in the case of *Penstemon centranthifolius* (Scrophulariaceae) (Mitchell & Shaw, 1993), it is accepted that environmental factors are *generally of more importance* within this context. Individual fecundities will depend more upon the environmental influence of variable traits than upon selection on fixed traits; for example, Herrera (1993) showed that, although there was apparent phenotypic selection on a range of floral traits in *Viola cazorlensis* (Violaceae), any potential effect of selection was overridden by the positive relationship between plant size and seed output. Plant size, in turn, was influenced by the substrate in which the plant grew, and Herrera's conclusion was that "...selection on the floral phenotype...may be largely irrelevant in evolutionary terms because other ecological factors are much more important determinants of fitness differences among plants". Thus, larger plants can dominate the gene pool of a population by their greater seed output and pollen donation, swamping any selection which might otherwise occur. The corollary of this is that selection would be expected to favour those traits which increase an individual's size, relegating the importance of selection by pollinators or seed predators. Also of less importance might be any selection for resource use optimisation, if said selection were concerned with trade-offs between reproductive components such as number of fruit per infructescence, number of seeds per pod and seed weight, rather than allocation to growth *versus* reproduction or defense. Thus, there may be no reason to suppose that any kind of trade-off optimisation of these characters has evolved; they are simply not as important to individual fecundity as plant size.

References

Abrahamson, W.G. & H. Caswell (1982) On the comparative allocation of biomass, energy and nutrients in plants. Ecology **63**: 982-991

Addicott, L.J.F. (1986) Variation in the costs and benefits of mutualism; the interaction between yuccas and yucca moths. Oecologia **70**: 486-494

Andersen, A.N. (1988) Insect seed predators may cause far greater losses than they appear to. Oikos **52**: 337-340

Andersen, A.N. (1989) How important is seed predation to recruitment in stable populations of long-lived perennials? Oecologia **81**: 310-315

Andersson, S. & B. Widén (1993) Pollinator-mediated selection on floral traits in a synthetic population of *Senecio integrifolius* (Asteraceae). Oikos **66**: 72-79

Antrobus, S. (1992) Ecology and genetics of colonising and established populations of *Primula veris*. Ph.D. Thesis, Oxford Polytechnic.

Augspurger, C.K. (1981) Reproductive synchrony of a tropical shrub: experimental studies of on effects of pollinators and seed predators on *Hybanthus prunifolius* (Violaceae). Ecology **62**: 775-788

Augspurger, C.K. (1983) Phenology, flowering synchrony and fruit set of six neotropical shrubs. Biotropica **15**: 257-267

Barth, F.G. (1991) Insects and Flowers: the Biology of a Partnership. Princeton University Press, U.S.A

Barrett, S.C.H. (1988) The evolution, maintenance, and loss of self-incompatibility systems. *in* Lovett Doust, J. & L. Lovett Doust (eds) Plant Reproductive Ecology: Patterns and Strategies. Oxford University Press, U.K.

Barrow, D.A. & R.S. Pickard (1984) Size-related selection of food plants by bumblebees. Ecological Entomology **9**: 369-373

Batiste, W.C. (1967) Biology of the Trefoil seed chalcid, *Bruchophagus kolobovae* Fedoseeva (Hymenoptera, Eurytomidae). Hilgardia **38**: 427-467

Bawa, K.S. (1983) Patterns of flowering in tropical plants *in* Jones, C.E. & R.J. Little (eds) Handbook of Experimental Pollination Biology. Scientific and Academic Editions, U.S.A.

Bazzaz, F.A. & D.D. Ackerly (1992) Reproductive allocation and reproductive effort in plants *in* M. Fenner (ed.) Seeds: The Ecology of Regeneration in Plant Communities. C.A.B., U.K.

Bazzaz, F.A., R.W. Carlson & J.L. Harper (1979) Contribution to the reproductive effort by photosynthesis of flowers and fruits. Nature **279**: 554-555

Bertin, R.I. (1988) Paternity in plants *in* Lovett Doust, J. & L. Lovett Doust (eds) Plant Reproductive Ecology: Patterns and Strategies. Oxford University Press, U.K.

Bierzychudek, P. (1981) Pollinator limitation of plant reproductive effort. The American Naturalist **117**: 838-840

Brady, N.C. (1990) The Nature and Properties of Soils. (10th ed.) Macmillan, U.S.A.

Brian, A.D. (1957) Differences in the flowers visited by four species of bumblebees and their causes. Journal of Animal Ecology **26**: 71-98

Brody, A.K. (1992a) Oviposition choices by a pre-dispersal seed predator (*Hylemya* sp.) I. Correspondence with hummingbird pollinators, and the role of plant size, density and floral morphology. Oecologia **91**: 56-62

Brody, A.K. (1992b) Oviposition choices by a pre-dispersal seed predator (*Hylemya* sp.) II. A positive association between female choice and fruit set. Oecologia **91**: 63-67

Brødsgaard, B. & I.R. Rasmussen (1990) Gene flow in the species *Lotus corniculatus* L. M.Sc. thesis, University of Copenhagen, Denmark.

Brown, V.K., M. Jepsen & C.W.D. Gibson (1988) Insect herbivory: effects on early old field succession demonstrated by chemical exclusion methods. Oikos **52**: 293-302

Brown, V.K., C.W.D. Gibson & P.M. Sterling (1990) The mechanisms controlling insect diversity in calcareous grasslands *in* Hillier, S.H., D.W.H. Walton & D.A. Wells (eds) Calcareous Grasslands - Ecology and Management. Bluntisham, U.K.

Bush, M.B. (1993) An 11 400 year paleoecological history of a British chalk grassland. Journal of Vegetation Science **4**: 47-66

Buzzell, R.I. & C.P. Wilsie (1964) Seed production potential of flowering types in birdsfoot trefoil. Crop Science **4**: 436-437

Calvo, R.N. & C.C. Horvitz (1990) Pollinator limitation, cost of reproduction, and fitness in plants: a transition-matrix demographic approach. American Naturalist **136**: 499-516

Carleton, A.E. & C.S. Cooper (1972) Seed size effect upon seedling vigour of three forage legumes. Crop Science **10**: 48-58

Carpenter, F.L. (1976) Plant-pollinator interactions in Hawaii: pollination energetics of *Metrosideros collina* (Myrtaceae). Ecology **57**: 1125-1144

Clapham, A.R., T.G. Tutin & D.M. Moore (1987) Flora of the British Isles (3rd ed.). Cambridge University Press, U.K.

Cochrane, V.W. (1958) Physiology of Fungi. John Wiley, U.K.

Compton, S.G.A. (1983) Studies of insects associated with *Lotus corniculatus* L. Ph.D. Thesis, University of Hull.

Crawford, T.J. (1984) What is a population? *in* B. Shorrocks (ed.) Evolutionary Ecology (23rd Symposium of The British Ecological Society). Blackwell Scientific Publications, U.K.

Crawley, M.J. (1992) Seed predators and plant population dynamics *in* Fenner, M. (ed) Seeds - The Ecology of Regeneration in Plant Communities. C.A.B. International, U.K.

Dafni, A. (1992) Pollination Ecology: a Practical Approach. IRL Press, U.K.

Darwin, C. (1876) The Effects of Cross and Self Fertilisation in the Vegetable Kingdom. Murray, U.K.

DeGrandi-Hoffman, G. & C.H. Collison (1982) Flowering and nectar secretion as they relate to honeybee foraging activity in birdsfoot trefoil (*Lotus corniculatus*). Journal of Apicultural Research **21**: 199-207

de Jong, T.J. & P.G.L. Klinkhamer, (1989) Size dependence of sex-allocation in monocarpic, hermaphroditic plants. Functional Ecology **3**: 201-206

de Jong, T.J. & P.G.L. Klinkhamer, (1991) Early flowering in *Cynoglossum officinale* L. constraint or adaptation? Functional Ecology **5**: 750-756 de Jong, T.J., P.G.L. Klinkhamer & M.J. van Staalduinen (1992) The consequences of pollination biology for selection of mass or extended blooming. Functional Ecology **6**: 606-615

de Jong, T.J., N.M. Waser & P.G.L. Klinkhamer (1993) Geitonogamy: the neglected side of selfing. Trends in Ecology and Evolution **8**: 321-325

De Ridder, F. (1990) Demografische variatie en adaptieve waarde van het reproduktief gedrag van de Kleine Zonnedauw (*Drosera intermedia* Hayne) in vershchillende habitaten. Ph.D. Thesis, University of Antwerp.

Dobrofsky, S. & W.F. Grant (1980a) Electrophoretic evidence supporting self-incompatibility in *Lotus corniculatus*. Canadian Journal of Botany **58**: 712-716

Dudash, M.R. (1991) Plant size effects on female and male function in hermaphroditic *Sabatia angularis* (Gentianaceae). Ecology **72**: 1004-1012

Ehrlén, J. (1991) Why do plants produce surplus flowers? A reserve-ovary model. The American Naturalist **138**: 918-933

El Atta, H.A. (1993) The effect of *Caryedon serratus* Olivier (Col., Bruchidae) on viability and germination of seeds of *Acacia nilotica* (L. Willd. ex Del.) in the Sudan. Forest Ecology and Management **57**: 169-177

Ellison, R.L. & J.N. Thompson (1987) Variation in seed and seedlings size: the effects of seed herbivores on *Lomatium grayi* (Umbelliferae). Oikos **49**: 269-280

Endler, J.A. (1986) Natural Selection in the Wild. Princeton University Press, U.S.A.

English-Loeb, G.M. & R. Karban (1992) Consequences of variation in flowering phenology for seed head herbivory and reproductive success in *Erigeron glaucus* (Compositae). Ecologia **89**: 588-595

Ernst, W.H.O., D.J. Tolsma & J.E. Decelle (1989) Predation of seeds of *Acacia tortila* by insects. Oikos 54: 294-300

Evans, E.W., C.C. Smith & R.P. Gendron (1989) Timing of reproduction in a prairie legume: seasonal impacts of insects consuming flowers and seeds. Oecologia 78: 220-230

Feinsinger, P (1978) Interactions between plants and hummingbirds in a successional tropical community. Ecological Monographs 48: 269-287

Feinsinger, P. (1987) Effect of plant species on each other's pollination: Is community structure influenced? Trends in Ecology and Evolution 2: 123-126

Fowler, A.D. (1993) The effects of herbivory on regeneration in limestone grassland. Ph.D. Thesis, University of London.

Frankie, G.W. & W.A. Haber (1983) Why bees move among mass-flowering neotropical trees *in* Jones, C.E. & R.J. Little (eds) Handbook of Experimental Pollination Biology. Scientific and Academic Editions, U.S.A.

Frankie, G.W., P.A. Opler & K.S. Bawa (1976) Foraging behaviour of solitary bees: implications for outcrossing of a neotropical forest tree species. Journal of Ecology 64: 1049-1057

Franson, S.E. & M.F. Willson (1983) Seed predation and patterns of fruit production in *Asclepias syriaca* L. Oecologia 59: 370-376

Fussell, M. & S.A. Corbet (1992) Flower usage by bumble-bees: a basis for forage plant management. Journal of Applied Ecology 29: 451-465

Galen, C., T. Gregory & L.F. Galloway (1989) Costs of self pollination in a self incompatible plant, *Polemonium viscosum*. American Journal of Botany 76: 1675-1680

Geber, M.A. (1985) The relationship of plant size to self-pollination in *Mertensia ciliata*. Ecology **66**: 762-772

Gentry, A.H. (1978) Anti-pollinators for mass-flowering plants? Biotropica **10**: 68-69

Gibson, C.W.D. (1986) Management history in relation to changes in the flora of different habitats on an Oxfordshire estate, England. Biological Conservation **38**: 217-232

Gibson, C.W.D., V.K. Brown & M. Jepsen (1987a) Relationships between the effects of insect herbivory and sheep grazing on seasonal changes in an early successional plant community. Oecologia **71**: 245-253

Gibson, C.W.D., T.A. Watt & V.K. Brown (1987b) The use of sheep grazing to recreate species-rich grassland from abandoned arable land. Biological Conservation **42**: 165-183

Gough, M.W. & R.H. Marrs (1990) A comparison of soil fertility between semi-natural and agricultural plant communities: implications for the creation of species-rich grassland on abandoned agricultural land. Biological Conservation **51**: 83-96

Gould, S.J. (1990) Wonderful Life: The Burgess Shale and the Nature of History. Hutchinson Radius, U.K.

Green, T.W. & I.G. Palmbald (1975) Effects of insect seed predators on *Astragalus cibarius* and *Astragalus utahensis* (Leguminosae). Ecology **56**: 1435-1440

Grieg, N. (1993) Predispersal seed predation on five *Piper* species in tropical rainforest. Oecologia **93**: 412-420

Grime, J.P. (1979) Plant Strategies and Vegetation Processes. Wiley, U.K.

Grime, J.P. & A.V. Curtis (1976) The interaction of drought and mineral nutrient stress in calcareous grassland. Journal of Ecology **64**: 975-988

Grime, J.P., J.G. Hodgson & R. Hunt (1988) Comparative Plant Ecology. Unwin Hyman Ltd, U.K.

Gross, R.S. & P.A. Werner (1983) Relationships among flowering phenology, insect visitors, and seed-set of individuals: experimental studies on four co-occurring species of goldenrod (*Solidago*: Compositae). Ecological Monographs **53**: 95-117

Guitián, J. & J.M. Sánchez (1992) Flowering phenology and fruit set of *Petrocoptis grandiflora* (Caryophyllaceae). International Journal of Plant Science **153**: 409-412

Haig, D. & M. Westoby (1988a) Inclusive fitness, seed resources, and maternal care *in* Lovett Doust, J. & L. Lovett Doust (eds) Plant Reproductive Ecology: Patterns and Strategies. Oxford University Press, U.K.

Haig, D. & M. Westoby (1988b) On limits to seed production. The American Naturalist. **131**: 757-759

Hainsworth, F.R., L.L. Wolf & T. Mercier (1984) Pollination and pre-dispersal seed predation: net effects on reproduction and inflorescence characteristics in *Ipomopsis aggregata*. Oecologia **63**: 405-409

Halevy, G. (1974) Effects of gazelles and seed beetles (Bruchidae) on germination and establishment of *Acacia* species. Israel Journal of Botany **23**: 120-126

Harper, J.L. (1967) A darwinian approach to plant ecology. Journal of Ecology **55**: 247-270

Harper, J.L. (1977) Population Biology of Plants. Academic Press, U.K.

Hawes, C. (1988) Subcellular localization of macromolecules by microscopy *in* Shaw, C.H. (ed) Plant Molecular Biology: A Practical Approach. IRL Press, U.K.

Heinrich, B. (1975) Energetics of pollination. Annual Review of Ecology and Systematics **6**: 139-170

Heinrich, B. (1976) The foraging specializations of individual bumblebees. Ecological Monographs **46**: 105-128

Heinrich, B. & P.H. Raven (1972) Energetics and pollination. Science **176**: 597-602

Herrera, C.M. (1984) Selective pressures on fruit seediness: differential predation of fly larvae on the fruits of *Berberis hispanica.* Oikos **42**: 166-170

Herrera, C.M. (1993) Selection on floral morphology and environmental determinants of fecundity in a hawk moth-pollinated violet. Ecological Monographs **63**: 251-275

Hodgkin, K.C. & Quinn, J.A. (1978) Environmental and genetic control of reproduction in *Danthonia caespitosa* populations. Australian Journal of Botany **26**: 351-364

Hunter, M.D., T. Ohgushi & P.W. Price (eds) (1992) Effects of Resource Distribution on Animal-Plant Interactions. Academic Press, U.S.A.

Janzen, D.H. (1971) Seed predation by animals. Annual Review of Ecology and Systematics **2**: 465-492

Janzen, D.H. (1976a) Effect of defoliation on fruit bearing branches of the Kentucky Coffee Tree, *Gymnocladus dioicus* (Leguminosae). American Midland Naturalist **95**: 474-478

Janzen, D.H. (1976b) Why bamboos wait so long to flower. Annual Review of Ecology and Systematics **7**: 347-391

Janzen, D.H. (1979) How to be a fig. Annual Review of Ecology and Systematics **10**: 13-51

Jeffrey, D.W. & C.D. Pigott (1973) The response of grasslands on sugar limestone in Teesdale to application of phosphorous and nitrogen. Journal of Ecology **61**: 85-92

Jennersten, O., L. Berg & C. Lehman (1988) Phenological differences in pollinator visitation, pollen deposition and seed set in the sticky catchfly, *Viscaria vulgaris*. Journal of Ecology **76**: 1111-1132

Jones, C.E. & R.J. Little (eds) (1983) Handbook of experimental pollination biology. Scientific and Academic Editions, U.S.A.

Jones, D.A. (1962) Selective eating of the acyanogenic form of the plant *Lotus corniculatus* L. by various animals. Nature **193**: 1109-1110

Jones, D.A. (1977) On the polymorphism of cyanogenesis in *Lotus corniculatus* L. VII. The distribution of the cyanogenic form in western Europe. Heredity **39**: 27-44

Jones, D.A. & R. Turkington (1986) Biological Flora of the British Isles: *Lotus corniculatus* L. Journal of Ecology
74: 1185-1212

Jones, D.A., S.G. Compton, T.J. Crawford, W.M. Ellis & I.M. Taylor (1986) Variation in the colour of the keel petals in *Lotus corniculatus* L. 3. Pollination, herbivory and seed production. Heredity **57**: 101-112

Kaul, R.B. (1979) Inflorescence architecture and flower sex ratio in *Sagittaria brevirostra* (Alismataceae). American Journal of Botany **66**: 1062-1066

Klinkhamer, P.G.L. & T.J. de Jong (1990) Effects of plant size, plant density and sex differential nectar reward on pollinator visitation in the protandrous *Echium vulgare* (Boraginaceae). Oikos 57: 399-405

Klinkhamer, P.G.L. & T.J. de Jong (1993a) Phenotypic gender in plants: effects of plant size and environment on allocation to seeds and flowers in *Cynoglossum officinale*. Oikos 67: 81-86

Klinkhamer, P.G.L. & T.J. de Jong (1993b) Attractiveness to pollinators: a plant's dilemma. Oikos 66: 180-184

Klinkhamer, P.G.L., T.J. de Jong & G-J. de Bruyn (1989) Plant size and pollinator visitation in *Cynoglossum officinale*. Oikos 54: 201-204

Klinkhamer, P.G.L., E. Meelis, T.J. de Jong & J. Weiner (1992) On the analysis of size-dependent reproductive output in plants. Functional Ecology 6: 308-316

Kochmer, J.P. & S.N. Handel (1986) Constraints and competition in the evolution of flowering phenology. Ecological Monographs 56: 303-325

Lloyd, D.G. (1980) Sexual strategies in plants I. An hypothesis of serial adjustment of maternal investment during one reproductive session. New Phytologist 86: 69-79

Lloyd, D.G., C.J. Webb & R.B. Primack (1980) Sexual strategies in plants II. Data on the temporal regulation of maternal investment. New Phytologist 86: 81-92

Louda, S.M. (1982) Distribution ecology: variation in plant recruitment over a gradient in relation to insect seed predation. Ecological Monographs 52: 25-41

Lovett Doust, J. (1989) Plant reproductive strategies and resource allocation. Trends in Ecology and Systematics 4: 230-234

Lovett Doust, J. & L. Lovett Doust (eds) (1988) Plant Reproductive Ecology: Patterns and Strategies. Oxford University Press, U.K.

Lyons, E.E. & T.W. Mully (1992) Density effects on flowering phenology and mating potential in *Nicotiana alata.* Oecologia **91**: 93-100

MacDonald, L.H. (1946) Birdsfoot trefoil (*Lotus corniculatus* L.). Its characteristics and potentialities as a forage legume. Memoirs of the Cornell University Agriculture Experimental Station no. 261.

Marquis, R.J. (1988) Phenological variation in the neotropical understory shrub *Piper arieianum*: causes and consequences. Ecology **69**: 1552-1565

Marshall, C. & J. Grace (eds) (1992) Fruit and Seed Production: Aspects of Development, Environmental Physiology and Ecology. Cambridge University Press, U.K.

Mazer, S.J., A.A. Snow & M.L. Stanton (1986) Fertilization dynamics and parental effects upon fruit development in *Raphanus raphanistrum: consequences* for seed size variation. American Journal of Botany **73**: 500-511

McKersie, B.D., D.T. Tomes & S. Yamamoto (1981) Effect of seed size on germination, seedling vigour, electrolyte leakage, and establishment of bird's-foot trefoil (*Lotus corniculatus* L.) Canadian Journal of Plant Science **61**: 337-343

Melampy, M.N. (1987) Flowering phenology, pollen flow and fruit production in the andean shrub *Befaria resinosa.* Oecologia **73**: 293-300

Michaels, H.J., B. Benner, A.P. Hartgerink, T.D. Lee, S. Rice, M.F. Wilson & R.I. Bertin (1988) Seed size variation: magnitude, distribution, and ecological correlates. Evolutionary Ecology **2**: 157-166

Miri, R.K. & J.S. Bubar (1966) Self-incompatibility as an out-crossing mechanism in birdsfoot trefoil (*Lotus corniculatus*). Canadian Journal of Plant Science **46**: 411-418

Mitchell, R.J. & R.G. Shaw (1993) Heritability of floral traits for the perennial wild flower *Penstemon centranthifolius* (Scrophulariaceae): clones and crosses. Heredity **71**: 185-192

Mitchley, J. (1988) Restoration of species-rich calcicolous grassland on ex-arable land in Britain. Trends in Ecology and Evolution **3**: 125-127

Molau, U., B. Eriksen & J.T. Knudsen (1989) Predispersal seed predation in *Bartsia alpina*. Oecologia **81**: 181-185

Moore, R.P. (1973) Tetrazolium staining for assessing seed quality *in* Heydecker, W. (ed) Seed Ecology. Butterworths, U.K.

Murrell, D.C., D.T. Tomes & R.W. Shuel (1982) Inheritance of nectar production in birdsfoot trefoil. Canadian Journal of Plant Science **62**: 101-105

Ohlson, M. (1988) Size-dependent reproductive effort in three populations of *Saxifraga hirculus* in Sweden. Journal of Ecology **76**: 1007-1016

Ollerton, J, & A.J. Lack (1992) Flowering phenology - an example of relaxation of natural selection? Trends in Ecology and Evolution **7**: 274-276

Pellmyr, O. (1992) The phylogeny of a mutualism: evolution and coadaptation between *Trollius* and its seed-parasitic pollinators. Biological Journal of the Linnean Society **47**: 337-365

Pettersson, M.W. (1992) Pollination, oviposition and seed predation by flower visiting insects in bladder campions (*Silene vulgaris* S.L., Caryophyllaceae). Ph.D. Thesis, Uppsala University.

Pors, B. & P.A. Werner (1989) Individual flowering time in a goldenrod (*Solidago canadensis*): field experiment shows genotype more important than environment. American Journal of Botany **76**: 1681-1688

Primack, R.B. (1980) Variation in the phenology of natural populations of montane shrubs in New Zealand. Journal of Ecology **68**: 849-862

Primack, R.B. (1985) Patterns of flowering phenology in communities, populations, individuals, and single flowers *in* White, J. (ed) The Population Structure of Vegetation (Handbook of Vegetation Science III). Dr. W. Junk Publishers, Netherlands.

Primack, R.B. (1987) Relationships among flowers, fruits, and seeds. Annual Review of Ecology and Systematics **18**: 409-430

Proctor, M. & P. Yeo (1973) The Pollination of Flowers. Collins, London.

Prokopy, R.J., B.D. Roitberg & A.L. Averill (1984) Resource Partitioning *in* W.J. Bell & R.T. Cardé (eds) Chemical Ecology of Insects. Chapman and Hall, U.K.

Prŷs-Jones, O.E. & S.A. Corbet (1991) Bumblebees. (Naturalists' Handbooks 6.) 2nd ed. Cambridge University Press, U.K.

Pyke, G.H. (1991) What does it cost a plant to produce floral nectar? Nature **350**: 58-59

Ranta, E. & H. Lundberg (1980) Resource partitioning in bumblebees: the significance of differences in proboscis length. Oikos **35**: 298-302

Rasmussen, I.R. & B. Brødsgaard (1992) Gene flow inferred from seed dispersal and pollinator behaviour compared to DNA analysis of restriction site variation in a patchy population of *Lotus corniculatus* L. Oecologia **89**: 277-283

Rathke, B. & E.P. Lacey (1985) Phenological patterns of terrestrial plants. Annual Review of Ecology and Systematics **16**: 179-214

Raven, P.H. (1983) *Foreword to* Real, L. (ed.) Pollination Biology. Academic Press, London.

Real, L. (ed) (1983) Pollination Biology. Academic Press, London.

Reekie, E.G. & Bazzaz, F.A. (1987a) Reproductive effort in plants. I. Carbon allocation to reproduction. American Naturalist **129**: 876-896

Reekie, E.G. & F.A. Bazzaz (1987b) Reproductive effort in plants. 2. Does carbon reflect the allocation of other resources? American Naturalist **129**: 897-906

Reekie, E.G. & Bazzaz, F.A. (1987c) Reproductive effort in plants. 3. Effect of reproduction on vegetative activity. American Naturalist **129**: 907-919

Robertson, A.W. (1992) The relationship between floral display size, pollen carryover and geitonogamy in *Myosotis colensoi* (Kirk) Macbride (Boraginaceae). Biological Journal of the Linnean Society **46**: 333-349

Robertson, A.I., R. Giddins & T.J. Smith (1990) Seed predation by insects in tropical mangrove forests: extent and effects on seed viability and the growth of seedlings. Oecologia **83**: 213-219

Rorison, I.H. (1990) Soils, mineral nutrition and climate. *in* Hillier, S.H., D.W.H. Walton & D.A. Wells (*eds*) Calcareous Grasslands Ecology and Management. Bluntisham Books, U.K.

Sallabanks, R. & S.P. Courtney (1992) Frugivory, seed predation, and insect-vertebrate interactions. Annual Review of Entomology **37**: 377-400

Samson, D.A. & K.S. Werk (1986) Size-dependant effects in the analysis of reproductive effort in plants. American Naturalist **127**: 667-680

Sandha, G.S., B.E. Twamley & B.R. Christie (1977) Analysis of quantitative variability for seed yield and related characters in *Lotus corniculatus* L. cv. Leo. Euphytica **26**: 113-122

Schmid-Hempel, P. & Speiser, B. (1988) Effects of inflorescence size on pollination in *Epilobium angustifolium.* Oikos **53**: 98-104

Schmitt, J. (1983) Individual flowering phenology, plant size, and reproductive success in *Linanthus androsaceus*, a California annual. Oecologia **59**: 135-140

Seaney, R.R. & P.R. Henson (1970) Birdsfoot trefoil. Advances in Agronomy **22**: 119-157

Seavy, S.R. & K.S. Bawa (1986) Late-acting self-incompatibility in angiosperms. The Botanical Review **52**: 195-219

Siemens, D.H., C.D. Johnson & K.J. Ribardo (1992) Alternative seed defense mechanisms in congeneric plants. Ecology **73**: 2152-2166

Snow, D.W. (1965) A possible selective factor in the evolution of fruiting seasons in tropical forest. Oikos **15**: 274-281

Southwick, E.E. (1984) Photosynthate allocation to floral nectar: A neglected energy investment. Ecology **65**: 1775-1779

Stearns, S.C. (1977) The evolution of life history traits: a critique of the theory and a review of the data. Annual Review of Ecology and Systematics **8**: 145-171

Stephenson, A.G. (1979) An evolutionary examination of the floral display of *Catalpa speciosa* (Bignoniaceae). Evolution **33**: 1200-1209

Stephenson, A.G. (1982) Flower and fruit abortion: proximate causes and ultimate functions. Annual Review of Ecology and Systematics **12**: 253-279

Stephenson, A.G. (1982) When does outcrossing occur in a mass-flowering plant? Evolution **36**: 762-767

Stephenson, A.G. (1984) The regulation of maternal investment in an indeterminate flowering plant (*Lotus corniculatus*). Ecology **65**: 113-121

Stephenson, A.G. (1992) The regulation of maternal investment in plants *in* Marshall, C. & J. Grace (eds) Fruit and Seed Production: Aspects of Development, Environmental Physiology and Ecology. Cambridge University Press, U.K.

Stephenson, A.G. & J.A. Winsor (1986) *Lotus corniculatus* regulates offspring quality through selective fruit abortion.
Evolution **40**: 453-458

Thom son, J.D. (1985) Pollination and seed set in *Diervilla lonicera* (Caprifoliaceae): temporal patterns of flower and ovule deployment. American Journal of Botany **72**: 737-740

Ting, I.P. (1982) Plant Physiology. Addison-Wesley, U.S.A.

Turkington, R. & G.D. Franko (1980) The biology of Canadian weeds. 41. *Lotus corniculatus* L. Canadian Journal of Plant Sciences **60**: 965-979

Venable, D.L. (1992) Size-number trade-offs and the variation of seed size with plant resource status. The American Naturalist **140**: 287-304

Verkaar, H.J. & A.J. Schenkeveld (1984) On the ecology of short-lived forbs in chalk grasslands: semelparity and seed output of some species in relation to various levels of nutrient supply. New Phytologist **98**: 673-682

Waller, D.M. (1982) Factors influencing seed weight in *Impatiens capensis* (Balsaminaceae). American Journal of Botany **69**: 1470-1475

Waller, D.M. (1993) How does mast-fruiting get started? Trends in Ecology and Evolution **8**: 122-123

Waser, N.M. (1978) Competition for hummingbird pollination and sequential flowering in two Colorado wildflowers. Ecology **59**: 934-944

Waser, N.M. (1979) Pollinator availability as a determinant of flowering time in Ocotillo (*Fouquieria splendens*). Oecologia **39**: 107-121

Waser, N.M. & L.A. Real (1979) Effective mutualism between sequentially flowering plant species. Nature **281**: 670-672
Watson, M.A. (1984) Developmental constraints: effect on population growth and patterns of resource allocation in a clonal plant. American Naturalist **123**: 411-426

Watson, M.A. (1986) Integrated physiological units in plants. Trends in Ecology and Systematics **1**: 119-123

Watson, M.A. & B.B. Casper (1984) Morphogenetic constraints on patterns of carbon distribution in plants. Annual Review of Ecology and Systematics **15**: 233-259

Weiner, J. (1988) The influence of competition on plant reproduction *in* Lovett-Doust, J. & L. Lovett-Doust (eds) Plant Reproductive Ecology: Patterns and Strategies. Oxford University Press, U.K.

Weis, A.E. & D.R. Campbell (1992) Plant genotype: a variable factor in insect-plant interactions *in* Hunter, M.D., T. Ohgushi & P.W. Rice (eds) Effects of Resource Distribution on Plant-Animal Interactions. Academic Press, U.K.

Widén, B. (1991) Phenotypic selection on flowering phenology in *Senecio integrifolius*, a perennial herb. Oikos **61**: 205-215

Willis, A.J. (1963) Braunton Burrows: the effects on the vegetation of the addition of mineral nutrients to the dune soils. Journal of Ecology **51**: 353-374

Willson, M.F. & B.J. Rathcke (1974) Adaptive design of the floral display in *Asclepias syriaca* L. The American Midland Naturalist **92**: 47-57

Willson, M.F., L.J. Miller & B.J. Rathcke (1979) Floral display in *Phlox* and *Geranium*: adaptive aspects. Evolution **33**: 52-63

Woodell, S.R.J. & J. Steel (1990) Seed bank and vegetation development of abandoned arable land, Upper Seeds Wytham. Report No. OX/0201/B, Bioscan (UK) Limited. Commissioned by the Nature Conservancy Council, U.K.

Wyatt, R. (1980) The reproductive biology of *Asclepias tuberosa*: I. Flower number, arrangement, and fruit-set. New Phytologist **85**: 119-131

Wyatt, R. (1982) Inflorescence architecture: how flower number, arrangement, and phenology affect pollination and fruit-set. American Journal of Botany **69**: 585-594

Wyatt, R. (ed) (1992) Ecology and evolution of plant reproduction: new approaches. Routledge, Chapman & Hall, Inc., U.S.A.

Zar, J.H. (1984) Biostatistical Analysis (2nd ed.). Prentice Hall, USA.

Zimmerman, M. (1980) Reproduction in *Polemonium*: pre-dispersal seed predation. Ecology **61**: 502-506

Zimmerman, M. (1988) Nectar production, flowering phenology, and strategies for pollination *in* Lovett-Doust, J. & L. Lovett-Doust (eds) Plant Reproductive Ecology: Patterns and Strategies. Oxford University Press, U.K.

Zimmerman, M. & R.S. Gross (1984) The relationship between flowering phenology and seed set in an herbaceous perennial plant, *Polemonium foliosissimum* Gray. The American Midland Naturalist **111**: 185-191

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