A BIOASSAY TEST OF THE INHIBITION OF SEED GERMINATION BY FLESHY FRUIT TISSUES

COLIN BURROWS

Introduction

Numerous tests of native New Zealand plant species have shown that, if the seeds are left within the fleshy pericarp of their fruit (tissue derived from the ovary wall, that encloses the seeds) they do not germinate, or germinate at a very slow rate, and in low numbers (e.g. Burrows, 1995a; 1995b; 1996; 1999). On the other hand, for many species, if seeds from the same batch have the flesh cleaned off and are soaked overnight in tap water, they germinate relatively easily.

The inhibitory effect of fruit tissues on seed germination has been observed for some horticultural species, e.g. in tomatoes and apples. It has also been recorded from some dry-fruited species (see Bradbeer, 1988; Mayer & Poljakoff-Mayber, 1989). The causes of the inhibition are generally put down to the presence of particular compounds, including coumarin, ferulic acid, and the growth inhibitor abscissic acid. Some plant physiologists have suggested that there are other causes for the germination inhibition, for example exclusion of oxygen by seed coats or pericarp, or the osmotic influences of strong concentrations of sugars in the fruit tissues. However, the chemical inhibition hypothesis is the most popular one to account for these effects, at present.

In a study of *Melicytus ramiflorus* (mahoe) seed germination Partridge & Wilson (1990) found that a brown solution leached from the seeds when they were soaked in water. This solution prevented germination of the *Melicytus* seeds, as well as those of *Fuchsia excorticata* (kotukutuku), *Kunzea ericoides* (kanuka) and two introduced plants *Bromus unioloides* (prairy grass) and *Spergula arvensis* (spurrey). These results will be considered later, in more detail. Testing the effects of an extract from one species by its influence on the growth of others is a form of *bioassay*. My own observations show that, although water-soluble compounds leach from the seeds of *Melicytus ramiflorus*, and other native

fleshy-fruited species, the fleshy tissues surrounding the seeds also contain such materials, so the pericarps, rather than the seed coats appear to be the primary seat of the inhibitory effect (see Burrows 19971, 1997b). As seed germination in the fruit on their parents would be detrimental for almost all species, inhibition by compounds in the fruit tissues has the important function of preventing this viviparous germination. In nature when birds or other animals swallow fleshy fruit, the pericarp tissues, and their inhibitory effect on germination, are removed.

In 1990-91 I did some simple bioassay tests on the influences of fruit tissue extracts from several native species. The actual bioassay was the response of lettuce seeds to the plant extract. Lettuce seeds are convenient for this purpose because they germinate within 24 hours when moistened and kept warm, the seedlings emerge from their enclosing tissues, and their sizes and other responses are very uniform.

Three experiments were done. The first aimed to examine the effects of leachate from fleshy fruit tissues of two species on rate of germination of lettuce seeds and growth of the seedlings. The second was similar but examined the effects of leachate from fleshy pericarp and separated seeds of three species. The third was similar to the second, but two species have fleshy fruit; the third has dry pericarp. The overall aim was to establish whether or not fruit tissue can have an inhibitory effect on seed germination, using rapidly-germinating lettuce seeds as a bioassay test subject.

Methods

Experiment 1, 1990

On 21 February 1990 fifteen freshly-collected ripe fruit of each of *Aristotelia serrata* and *Melicytus ramiflorus* were squashed, the seeds removed and the flesh placed in new plastic petri dishes each containing a filter paper. Ten ml of tap water were placed in each of the dishes (two replicates for each species, as well as separate dishes for the untreated controls). These preparations were left to stand for 24 hours. Then, on 22 February 1990, 25 lettuce seeds (Watkins Great Lakes), purchased new in 1990, were placed in each dish, including the controls. The dishes were kept on a bench beside a window in a cool room and examined daily to record the rate of germination and other relevant phenomena.

Experiment 2, 1991

On 1 April 1991 fifteen freshly-collected ripe fruit of each of *Aristotelia serrata, Coprosma robusta* and *Hedycarya arborea* were squashed. The seeds and flesh were separated and each leached separately in a petri dish with a filter paper, in 10 ml of water, for 48 hours (two replicates of each, with separate dishes for controls). On 3 April 1991, 20 lettuce seeds (Watkins Great Lakes), newly purchased in 1991, were placed in each of the dishes which were sited as before, and monitored for germination and other relevant phenomena.

Experiment 3, 1991

On 7 April 1991 fifteen freshly-collected ripe fruit of each of *Griselinia littoralis* and *Melicytus ramiflorus* were squashed and the seeds separated from the flesh. Fifteen ripe capsules of *Pittosporum tenuifolium* were opened and the seeds removed. Then the pericarp tissues and seeds of each species were separately leached for 96 hours, in petri dishes with filter paper, 10 mls of water for *Griselinia* and *Melicytus* and for *Pittosporum* seeds, 20 mls of water for the dry capsules of *Pittosporum* (two replicates of both pericarp and seeds for each species, as well as separate dishes for the controls). On 11 April 1991 20 Watkins Great Lakes lettuce seeds (same batch as for Experiment 2) were placed in the dishes and monitored for germination and other relevant phenomena.

Results and Analysis

Experiment 1 (started 22 February 1990)

When the experiment was set up abundant brown leachate was evident in the *Aristotelia* preparation. A less opaque dark leachate appeared in the *Melicytus* preparation. Germination of all seeds in the control replicates was quick. Within four days about half of the seedlings grew to as much as 20 mm long; a few were only 10 mm long, and the rest between these sizes (Table 1). In the *Aristotelia* and *Melicytus* treatments the total germination success was much poorer and growth of the seedlings was very retarded. This

applied mainly to the root and hypocotyl elongation. Some lettuce seedlings in *Aristotelia* replicate (b) were very pale. In *Melicytus* replicate (a) some seedlings had very stunted roots (less than 0.5 mm long). Differences between replicates are evident (Table 1); in the *Melicytus* replicate (b) no germination occurred at all. This suggests that concentrations of inhibitors differed markedly between replicates as the control results for each replicate were uniform.

Table 1 Number of lettuce seeds (n = 25) that germinated (in brackets, range of seedling lengths, in mm, to nearest 0.5 mm)

		Aristotelia		*Melicytus*		Control	
Date	Treatment	a	b	a	b	a	b
23/2/90	Pericarp	-	4 (0.5)	1 (0.5)	-	25 (1-1.5)	24 (1-1.5)
26/2/90	Pericarp	1 (6)	11 (1-3)	13 (0.5-1)	-	25 (10-20)	24 (10-20)

Experiment 2 (started 3 April 1991)

When this experiment was set up, there was abundant brown leachate in the *Aristotelia* and *Hedycarya* preparations and paler brown leachate in the *Coprosma* preparation. Within two days of the start of the experiment germination was completed in the control. No *Aristotelia* or *Coprosma* pericarp treatment seeds had germinated and only one seed in the *Hedycarya* pericarp treatment (Table 2). The strong inhibitory effect of *Aristotelia* pericarp continued throughout the seven days' duration of the experiment although about half the seeds in one replicate had germinated by the fifth day. The pericarp leachate initially slowed lettuce seed germination considerably in both the *Coprosma* and *Hedycarya* treatments but, by seven days the degree of success was not significantly different from that in the control. A more noticeable effect of pericarp leachate in all of the treatments was the stunting of lettuce seedling growth, especially affecting hypocotyl and root elongation and causing suppression of root hairs.

For *Coprosma* and *Hedycarya* there was no apparent effect on germination rate of lettuce seeds in seed leachate, compared with that in the control. However, there was a distinct slowing of the rate in the *Aristotelia* preparation: 75% had germinated after seven days, (control 95%). The length growth of the lettuce seedlings was also retarded by *Aristotelia*

seed leachate, but little if at all in the other two preparations. The same pattern occurred for limitation of root hair development (none in the *Aristotelia* preparation, but abundant root hairs in the *Coprosma* and *Hedycarya* preparations). There is an indication that *Hedycarya* seed leachate actually stimulated growth of lettuce seedlings.

Table 2 Number of lettuce seeds (n = 20) that germinated (in brackets, range of seedling lengths, in mm, to nearest 0.5 mm) and occurrence of root hairs*

		Aristotelia			*Coprosma*			*Hedycarya*			Control		
Date	Treatment	a	b	Root hairs	a	b	Root hairs	a	b	Root hairs	a	b	Root hairs
4/4/91	Pericarp	0	0		0	0		1(0.5)	0		9(1)	11(1)	1
	Seeds	0	0		7(1)	10(1)	0	9(1)	10(1)	1			
5/4/91	Pericarp	0	0		4(0.5-1)	4(0.5-1)	0	8(0.5-3)	5(0.5-3)	0	19(3-5)	19(3-5)	2
	Seeds	13(0.5-1)	5(0.5-1)	0	18(2-5)	20(2-5)	2	18(4-8)	18(4-8)	2			
7/4/91	Pericarp	0	9(1-1.5)	0	18(1-4)	16(1-4)	0	12(0.5-2)	15(0.5-2)	0	19(15-20)	19(15-20)	2
	Seeds	15(0.5-2)	15(0.5-2)	0	18(1.5-2)	20(1.5-2)	2	18(25-40)	18(25-40)	2			
10/4/91	Pericarp	0	12(1-1.5)	0	18(1-5)	16(1-5)	0	12(1-2.5)	15(1-2.5)	0	19(30-40)	19(30-50)	2
	Seeds	15(0.5-2)	15(0.5-2)	0	18(30-40)	20(30-40)	2	18(30-50)	18(30-50	2			
* 0 – none; 1 – some; 2 very abundant													

Experiment 3 (started 11 April 1991)

When this experiment was set up, abundant brown leachate was evident in the *Griselinia* and *Pittosporum* pericarp preparations and less opaque dark leachate in the *Melicytus* preparation. Two days after the test started most of the control seeds had germinated. Only about half of the lettuce seeds in the *Melicytus* and more than half in the *Griselinia* pericarp leachate treatments had germinated, and none in the *Pittosporum* treatment. For this last species the inhibitory effect was complete for the duration of the experiment. However, by the end of the experiment on 15 April 1991 relatively high proportions of the lettuce seeds had germinated in the other two treatments (83% for *Melicytus*, 75% for *Griselinia*, 93% in the control) (Table 3).

Overall growth of lettuce seedlings in pericarp leachate was noticeably less than in the control and this was especially manifest in stunting of the roots (Table 3). Over the period of the experiment about half of the seedlings in the control set grew to well over twice the length of those in pericarp leachate from either *Melicytus* or *Griselinia*. Also root hair development was prevented in these two treatments. The cotyledons of many lettuce seedlings in the leachate from each of these species were white at the end of the experiment. No effect of *Melicytus* seed leachate could be discerned on lettuce seed germination rate or root hair development near the beginning of the experiment. There was, however, some slight retardation of germination and growth by *Griselinia* seed leachate and, at first, by *Pittosporum* seed leachate. After six days the retardation effect had disappeared (i.e. seedlings had appeared and elongated). Root hair development was prevented altogether by *Griselinia* seed leachate and diminished by *Pittosporum* leachate (Table 3).

Compared with the growth of lettuce seedlings in the control set, after one day there was a slight indication of stimulation of length growth of seedlings in leachate from seeds of the three tree species. By the end of the experiment, seedlings in the *Griselinia* treatment showed signs of some growth retardation, but those in the *Melicytus* and *Pittosporum* treatments seedling sizes were similar to those in the control.

Table 3 Numbers of lettuce seeds (n = 20) that germinated (in brackets, range of seedling lengths, in mm, to nearest 0.5 mm) and occurrence of root hairs*

		Melicytus			*Griselinia*			*Pittosporum*			Control		
Date	Treatment	a	b	Root hairs	a	b	Root hairs	a	b	Root hairs	a	b	Root hairs
13/4/91	Pericarp	8(0.5)	10(0.5)	0	11(0.5-1)	16(0.5-1)	0	-	-		17(0.5	19(0.5)	2
	Seeds	17(0.5-3)	19(0.5-3)	2	16(0.5-1)	13(0.5-1)	0	14(0.5-2)	16(0.5-2)	1			
14/4/91	Pericarp	14(0.5-1.5)†	14(0.5-1.5)†	0	14(0.5-2)†	16(0.5-2)†	0	-	-		18(2-4)	19(2-4)	2
	Seeds	18(2-8)	19(2-8)	2	16(2-3)	15(2-3)	0	14(0.5-2)	16(0.4-2)	1			
15/4/91	Pericarp	18(0.5-3)†	15(0.53)†	0	14(2-4)†	16(2-4)†	0	-	-		18(8-10)	19(8-10)	2
	Seeds	18(6-10)	19(5-10)	2	16(3-6)	15(3-5)	0	19(8-15)	18(8-12)	1			

* root hairs 0 = none; 1 = some; 2 = very abundant
† in these treatments the roots were all very stunted

Discussion

The three experiments, done in sequence, constitute a rough preliminary study of how the bioassay method can be applied to examining inhibitory effects of fruit tissues on seeds. More straightforward tests have included comparisons of germination behaviour of seeds of wild native plants, separated from fruit tissues and well washed, with seeds that have been left in the fruit tissues (Burrows, 1997b; 1999). However, the conditions of the present experiments were only crudely controlled. It was clear from the results of experiment 1 that uneven amounts of leachate were present in the paired replicates. To try to overcome this problem longer periods were allowed for leaching subsequently. Greater uniformity would probably be achieved by filtering the leachate from a given quantity of fruit and using standard volumes of the filtrate for treatment replicates, as was done by Partridge & Wilson (1990). Further investigation of the chemical nature of the filtrate is also desirable, but was beyond the scope of the study.

The experiments were done merely as a pilot study and the replication would need to be greater for properly valid conclusions and statistical analysis. Nevertheless, the results are reasonably clear-cut. They provide some clues to the ways that the chemicals present were affecting seeds and emerging seedlings. There was, in some cases, a degree of blocking of the germination process (contrasting markedly with what occurred in the untreated controls). There was also interference with normal growth processes that would allow root hair formation and rapid cell formation and expansion that allows roots and shoots to extend rapidly. Although these effects were being observed for seedlings (i.e., well after germination had occurred), it is assumed that growth processes in embryos, within the seed coats, would also be severely inhibited by the inhibitory compounds. It appears, also, that these compounds can interfere with biochemical processes affecting chlorophyll production, as was evident from the pale-coloured emerging seedlings noticed in some of the tests.

The test conditions with the tests operating for only a few days and water-diluted leachate (with the inhibitory compounds present in unknown concentrations) are unlike conditions experienced by seeds in fruit in natural conditions. When fruit are retained on their parents germination inhibitors must function for weeks or months. Also, in nature cells are usually not crushed (as they were in these tests) and the fleshy fruit of many species are not very

juicy. However, in the most succulent fruit, like those of *Coprosma* spp., or *Cordyline* spp., the seeds are bathed in a rather fluid solution. For *C. robusta* it is well known that seeds may remain, in fruit, on their parent for many months. In one case it was found that some seeds had germinated viviparously after fruit had remained over winter on the plant (Burrows, 1995b).

Partridge & Wilson's (1990) experiments involved leachate from large numbers (more than 400) seeds of *Melicytus ramiflorus*. This leachate inhibited the germination of leached *Melicytus* seeds, as well as those of *Coprosma robusta, Fuchsia excorticata*, and *Kunzea ericoides* (and two introduced species). These results and the conclusions from them contrast with those from the present experiments where leachate from seeds of a range of species generally showed negligible effects on germination of lettuce seeds, compared with the effects of leachate from pericarp. The differences between the results of these studies are probably explicable in terms of the relative concentrations of leachate involved. Relatively few seeds were leached to obtain the solutions for the present experiments. It appears, however, that materials present in the often relatively very large volume of enveloping pericarp is likely to have a more powerful inhibitory effect on seed germination than would the inhibitors present in each seed or seed coat. It seems likely that the inhibitory influences of compounds in seed coats originate in the surrounding maternal tissues (later to become the fruit) as ovaries and their contents mature.

Another set of important natural processes, are those which normally remove inhibitory influences derived from fruit tissues during and after seed dispersal. As fleshy fruit are eaten by birds or other animals most of the soluble inhibitory compounds present in them (and in seed coats) are simply removed by the enzymes of their digestive tracts. Dry fruit shed their seeds, which disperse in various ways. Attack by fungi and bacteria, using other sets of enzymes, will remove inhibitors present in their investing fruit tissues or seed coats.

The bioassay method with lettuce seeds shows promise as a means for rapid assessment of influence of inhibitors in fruit tissues on germination and early seedling development. However, more stringently controlled techniques, and analysis of the leachate to discover the nature of the inhibitory compounds, are needed to advance the methodology further. Perhaps, in future, someone will investigate the many fascinating aspects of this subject in more depth. Meanwhile, it is reasonable to assume that, by having chemical growth

inhibitors in their pericarp tissues, plants are protecting their future by preventing their seeds from germinating in the wrong place, at the wrong time.

Acknowledgments

I gratefully acknowledge much help from the Department of Plant & Microbial Sciences at the University of Canterbury. Funding for this and associated studies was provided by the Lotteries Grants Board.

References

Bradbeer, J.W. 1988. *Seed Dormancy and Germination*. Blackie, Glasgow.

Burrows, C.J. 1995a. Germination behaviour of the seeds of the New Zealand species *Fuchsia excorticata, Griselinia littoralis, Macropiper excelsum* and *Melicytus ramiflorus*. *New Zealand Journal of Botany 33*: 131-140.

Burrows, C.J. 1995b. Germination behaviour of the seeds of the New Zealand species *Aristotelia serrata, Coprosma robusta, Cordyline australis, Myrtus obcordata* and *Schefflera digitata*. *New Zealand Journal of Botany 33*: 257-264.

Burrows, C.J. 1996. Germination behaviour of the seeds of seven New Zealand woody plant species. *New Zealand Journal of Botany 34*: 355-367.

Burrows, C.J. 1997a. Reproductive ecology of New Zealand forests: 1. Natural seed storage phenomena. *New Zealand Natural Sciences 23*: 31-52.

Burrows, C.J. 1997b. Reproductive ecology of New Zealand forests: 2. Germination behaviour of seeds in varied conditions. *New Zealand Natural Sciences 23*: 53-69.

Burrows, C.J. 1999. Germination behaviour of seeds of the New Zealand woody species *Beilschmiedia tawa, Dysoxylum spectabile, Griselinia lucida* and *Weinmannia racemosa*. *New Zealand Journal of Botany 17:* 95-105.

Mayer, A.M., Poljakoff-Mayber, A. 1989. *The Germination of Seeds*. 4th Edn. Pergamon, Oxford.

Partridge, T.R., Wilson, M.D. 1990. A germination inhibitor in the seeds of mahoe (*Melicytus ramiflorus*). *New Zealand Journal of Botany 28*: 475-478.