THE POTENTIAL OF VETIVER GRASS TO PRODUCE FERTILE SEED WHEN USED FOR ROADSIDE STABILISATION IN COOK SHIRE

A report by John Hopkinson (Post-retirement Associate with DPI at Walkamin Research Station) to Paul Graham (Main Roads Dept, Cairns). 23 April 2002.

Summary. *Fears of the possibility of vetiver grass (Vetiveria zizanioides) becoming an environmental weed on Cape York Peninsula led to an investigation of the risks. A part of the investigation, reported here, was an assessment of the prospect of its uncontrolled spread by seed from plantings made for roadside stabilisation . Seed heads of plants of the apparently sterile cultivar Monto established vegetatively on road works in Cook Shire were screened for their ability to produce true seeds (caryopses). Samples averaging about 38 seed heads each had been collected for screening from eight sites on 2 occasions in March 2002 when the heads were ripe. In all, about 119,000 potentially fertile seed structures (sessile spikelets) were processed, but only 3 caryopses were found, a level of fertility of almost 1 in 40,000 or 0.0025%. The caryopses appeared to be structurally perfect, which was taken as an indication of the ability of Monto vetiver to produce viable seed in the conditions where it is grown, albeit with an extremely low level of success. The level of fertility is considered far too low to allow significant natural spread of vetiver by seed. The likelihood of fertility levels rising significantly in subsequent generations of plants derived from Monto vetiver, judged from overseas experience, is also very low. The only foreseeable risk of a rise in fertility of vetiver would come from the introduction of entirely different seeding types.*

Introduction

Vetiver grass (formerly *Vetiveria*, now *Chrysopogon, zizanioides*) has been used by the Queensland Main Roads Dept to stabilise new road work in Cook Shire. The cultivar Monto, widely believed to be sterile, was chosen for this purpose. Care was taken to use only authentic vegetative planting material. The usual method of propagating vetiver is to split existing plants and transplant the slips. The particular value of vetiver derives from the ability of its deeply penetrating roots of high-tensile strength to stabilise batters. Other perceived advantages of Monto vetiver over alternative soil-stabilising plants are that it is considered incapable of spreading by seed to places where it is unwanted, and (being unpalatable to stock) ought not to attract grazing animals to unfenced roadsides as do, for example, Indian couch and stylo.

Its use, however, has led to controversy. Some people are unconvinced of its inability to spread, and fear its becoming an environmental weed. It is not the business of this report to state the arguments for and against this view. Rather its business is to provide information on two questions important in these arguments, namely whether or not Monto vetiver is capable of production of seed in the places where it has so far been used in Cook Shire; and, if so, whether the amount of seed is enough to warrant concern over the risk of its unwanted spread. Given this information in conjunction with knowledge of vetiver's ability to spread vegetatively, those responsible for decisions about its further use ought to be able to make a balanced assessment of the weed risk.

Paul Graham recruited me to check seed for fertility. The requirement was to screen large numbers of potential seed-bearing structures to see if they did in fact form true seeds. It required the experience of a person familiar with the structure of grass seeds and their processing, and with access to the necessary equipment, to develop and apply quick, reliable screening methods. Having spent over 30 years in tropical pasture seed production, which included work on several grasses closely related to vetiver, and being still based at Walkamin RS which is fully equipped for small scale seed processing, I had the experience, the time, and access to the resources to do the screening. This is my report of the investigation. It is up to others more familiar with the general ecology of vetiver, particularly its ability to spread vegetatively, to take the matter further.

Seed structures and terminology

It is necessary at the outset to make clear what I am talking about. In particular, the specialised terminology needed to describe grass seed development must be explained. Popular use of the word "seed" is too loose for present purposes, and leads to endless confusion when applied to grasses. The reason is this. In most other plants, the progress of development from buds to flowers to fruits is obvious at a glance because each successive structure looks different from the last. But in grasses like vetiver the individual units of so-called seed (technical name *spikelets*) look externally alike at all three stages, and most people have no reason to discriminate between them and therefore call them all "seed". It is inside the spikelet, unseen, where the changes that matter take place. Inside, a single ovary may or may not be present which may or may not be fertilised by pollen. It may or may not then develop into a true seed, which may or may not mature into a viable seed. The actual true seed or *caryopsis*, if present, lies concealed within the covering structures of the so-called seed. In vetiver and most other typical grasses the caryopsis is tiny, but it can be accurately pictured as a miniature cereal grain, for the cereals are themselves also grasses and their grains caryopses.

The term *fertility* is used here to mean the number of caryopses formed expressed as a percentage of the number of ovaries (or of sessile spikelets) from which one might have formed.

Vetiver belongs to a group of grasses called the *Andropogoneae.* There are two main types of vetiver, a fertile wild type originally from northern India that seeds profusely, and a predominantly infertile domesticated type from southern India grown for its oil. The cultivar (cultivated variety) Monto is of the latter type. Infertility appears to be due to failure of pollination, which means that no caryopsis develops in the ovary.

The structure of vetiver seed heads is very much like that of its close relatives such as wild sorghums and Indian couch. Its spikelets occur in pairs on a loose many-branched seed heads. One of each pair is stalked and always barren; the other has no stalk and is potentially fertile (that is, it contains all the structures necessary to produce a caryopsis, in particular an ovary, from which the caryopsis develops). Here I shall call them the *barren* and *sessile spikelets* respectively.

Aim of investigation

My objective was primarily to look for caryopses, and to measure their frequency of occurrence in terms the number produced as a percentage of the number of sessile spikelets – that is to say, the number of ovaries from which a caryopsis might have developed.

Methods

Seed collection

Paul Graham organised the collection of seed at a time of year when seed was ripening and shedding – that is, as with most grasses that survive in the monsoonal savannas, at the end of the wet season. He made two collections two weeks apart (14 and 28 March 2002) at eight sites between the Palmer River, Cooktown and Laura. All were places where road work had been followed by vetiver planting. See Table 1 for details. He collected on average about 38 seed heads per sampling, which he put in cotton bags and delivered to Walkamin. Samples were dry on receipt and ready for processing.

Seed processing

Material from each bag was processed as a unit. It was first emptied on to a tray and the heads rubbed by hand until all loose spikelets had been detached. Any spikelets remaining attached would have been immature and lacking a viable caryopsis. At the base of each spikelet there develops a layer of cells that form a natural point for it to separate from the parent plant (the *abscission layer*). The abscission layer develops progressively with the caryopsis. In all grasses that we are familiar with (and therefore, it is assumed, vetiver) its development is advanced enough for the spikelet to be readily detached by rubbing before the caryopsis is fully mature.

The loose spikelets were sieved to remove fragments of stalk and weighed. A sub-sample of such material was taken, weighed, the total number of spikelets counted, and the number of spikelets per gram calculated. From this the total number of spikelets and the number of sessile spikelets (half the total, there being equal numbers of each of two types of spikelet) in each sample could be estimated.

Each sample was then blown in a laboratory blower designed to aspirate off light material from heavy. The blower consists of a fan blowing air through a cup with a mesh base and vertically up a tube before being exhausted. The sample is placed in the cup and blown at a pre-determined setting that governs airflow rate through the tube. A setting was chosen by trial and error, which blew off the barren spikelets and the lighter of the sessile ones. This reduced the sample size while removing material, which could not possibly contain a caryopsis (a caryopsis adds to the weight of a spikelet enough to reduce drastically its tendency to lift in an air stream). The remaining material was then rubbed between boards coated with ribbed rubber, which provides a convenient way of breaking up spikelets and releasing naked caryopses. The rubbed material was again blown to remove light fragments of spikelet and leave behind caryopses and the relatively few spikelets left intact. It was then placed under a low-power dissecting microscope (x 6 to x 40 magnification), where any loose caryopses were identified and counted and the contents all remaining spikelets checked. All caryopses recovered were loose, and no spikelets were found still to contain caryopses after rubbing. Caryopsis numbers were recorded.

Recognition of caryopses

I had never before seen a vetiver caryopsis, having had no previous reason to examine vetiver seed. It was a reasonable assumption that any caryopsis recovered would be of vetiver, since the samples contained only vetiver seed heads. Nevertheless seed is designed to get everywhere and absence of contamination can never be assumed, either from the bags or from my seed processing equipment, however much care is taken with cleanliness. I therefore consulted Bryan Simon, botanist and grass specialist at the Queensland Herbarium on the question of identification.

I had at the time retrieved one broken caryopsis, but was able to see that it was of a very unusual shape. A grass caryopsis is usually rather flat, shaped like a flat-bottomed boat, and when it lies on its back the embryo is in the position of a cockpit. This caryopsis was narrower than deep, with the embryo forming part of an upper ridge. The term for the shape is *laterally compressed*..

Bryan Simon confirmed that the spikelet of vetiver, along with other members of the genus *Chrysopogon* into which it has recently been placed, is laterally compressed. He believed that I could safely infer that the caryopsis would be likewise laterally compressed. Botanists see caryopses less often than do seed production agronomists, and I could find no one who could describe a vetiver caryopsis to me in detail. Nevertheless common sense convinces me that what I have are vetiver caryopses, the alternatives being too improbable to be credible, and I accept Bryan Simon’s advice.

Results

The 16 individual samples (from 8 sites x 2 sampling occasions) contained in all about 615 inflorescences (seed heads) yielding about 237, 600 loose spikelets of which about 118, 800 were sessile spikelets with all the structures necessary to produce a caryopses (Table 1). Out of these, 3 caryopses were recovered. One was intact and two were broken (the rubbing to extract from the spikelets introduces the risk of breakage). All caryopses were brown. The intact one had the appearance of being mature. It was 2.5 mm long, 0.4 mm wide, and 0.7 mm deep. It had a small but obvious eyespot (the “placental” area through which it was originally attached to the parent plant), a well-developed endosperm, and the scuffed remains of a normal embryo in the normal position. One of the broken caryopses had an intact embryo which appeared to be perfectly developed. The other appeared to be somewhat immature.

Discussion

Interpretation of results

I conclude that Monto vetiver in Cook Shire roadside plantings is indeed capable of producing fully developed seeds, but that the frequency with which it does so is extremely low. The best estimate of that frequency is one caryopsis in 40, 000 possible sites for caryopsis formation, or a 0.0025% success rate. The comparable usual success rate for a closely related sown pasture grass for which we have reliable figures is about 60% (Bisset blue grass, a cultivar of *Bothriochloa insculpta*). This figure is typical of most sown pasture grasses. The only native grass for which we have got comparable figures is the also fairly closely related Black Spear (*Heteropogon contortus*), which gave values between about 20 and 60%, depending on season. These figures serve to emphasise just how extremely low the seed set success rate of Monto vetiver was.

The wide range of sites sampled, the choice of two sampling occasions at times when seed could be expected to be ripe, and the very large number of plants, seed heads and seed structures screened, all lead me to think that it is safe to generalise, and to conclude that the behaviour recorded in the present exercise can be regarded as typical of that of Monto vetiver in the district in question. The conclusion could not, however, be extended to apply to other types or cultivars of vetiver without specific further testing.

No significance should be attached to the particular sites that produced the caryopses. Each occurrence can only be interpreted as a rare and random event which could have happened anywhere.

Whether or not the caryopses retrieved would have been capable of germinating and producing seedlings and plants was something I could not test. For one thing my methods caused them damage. For another there were far too few caryopses for any useful conclusion to be drawn on any tests that might have been conducted. I can only point out that the ones I recovered had been perfect in their development and were therefore probably potentially viable. I am prepared to believe that Monto vetiver has the capacity to produce the very occasional viable seed.

Past records of laboratory germination and tetrazolium tests on 42 different seed samples of Monto vetiver, harvested over 7 seasons from 26 sites covering almost all Queensland’s latitudinal range, plus sites in NSW, NT and Fiji, and conducted for Paul Truong by the Queensland Seed Testing Laboratory, showed nil viability. It can be assumed that in all over 33 000 seeds were individually tested. Samples of seed of fertile lines of vetiver were tested at the same time, providing a check on the testing methods, and found to produce viable seedlings. The QSTL result with Monto is not incompatible with the present records because, if the frequency of caryopses was the same as in the present samples, the chance of no caryopses at all being present in the spikelets tested at QSTL is quite high.

Preliminaries to discussion of implications

Before discussing the implications of the results, I must be sure that a reader is aware of the general background of success and failure of grass seed formation, development, shedding and survival; and of the hazards of germination and seedling establishment. I have no experience of vetiver itself but, basing expectations on the behaviour of closely related similarly perennial exotic grasses, I believe I can reconstruct these events with reasonable accuracy, and in the process illustrate some of the points that need to be made to get the extent of seed production of vetiver in perspective.

At the start of the wet season an established Monto vetiver plant puts out new shoots which eventually elongate and produce flower heads, the details of which have already been described. Each head includes numerous sessile spikelets, and each sessile spikelet has all the male and female parts needed for it to produce a seed.

The "seeds" of infertile types largely fail to produce caryopses. They nevertheless go through the process of ripening and shedding just as if they were doing something constructive, and thus give the impression of producing masses of seed. Most of what they produce, however, is inert chaff.

On the rare occasions when fertilisation is successful and a caryopsis is formed within a sessile spikelet, that caryopsis is subject to many hazards during its development. It may be parasitised by insect larvae (at least, some of its related natives, such as spear grass, are) or eaten by birds. The plant may experience unfavourable soil or weather conditions that curtail the maturation of the caryopsis and ultimately doom it. Despite these hazards, some caryopses may ripen successfully, and then be shed and fall to the ground. We assume (since this applies to all other grass seeds) that they then serve as a food source for birds, rodents, ants, and much else, over a period of at least nine months; and that many get eaten.

Even if it happens to lodge somewhere out of harm's way, a caryopsis then has to survive until the start of the next wet season. Many seeds simply age and die. Others fall down cracks in the soil to positions too deep for them to germinate from. Still others may germinate on early storms or in unsuitable positions, to die of drought before they can establish. The proportion of any grass seed population that survives to produce an established plant is thus small. Even in a prepared seedbed with everything in their favour, we expect over 90% of living pasture grass seeds to fail, and we choose our sowing rates accordingly. We have learnt by bitter experience to expect total failure of exotic pasture grass seeds (though not legumes) sown in undisturbed native vegetation. On heavily disturbed surfaces such as overgrazed pastures, graded roadsides, mine sites, roadworks, etc., which provide seedbeds intermediate between the previous two kinds, we expect intermediate results - some success, but only if very large numbers of good quality seeds are sown.

Even after a seedling has established the struggle is not over. The roots trying to function in a sward of adult plants tend to be beaten to scarce resources of water and nutrient and their shoots are likely at the same time to be starved of light by bigger plants. This is apt to restrict success to bare ground and gaps, and elsewhere innumerable seedlings perish as a result of stronger competition from earlier established, bigger, or better adapted neighbours.

The lesson we have learnt from all this is that an exotic grass which depends on seed to reproduce needs to produce massive numbers of fertile seeds to have any prospect of spreading. The lesson is the same whether we want the particular grass type to thrive or disappear.

Implications of interpretations

The frequency of occurrence of caryopses, even if viable, in the Cook Shire samples was so low as to be almost zero, and the chances of vetiver spreading to the point of becoming an environmental weed from seed from existing plants of Monto may reasonably be taken to be nil. If the plant is equally ill-adapted to spread naturally by vegetative means, the ecological disadvantage of so low a level of fertility are likely be devastating. In view of the levels of fertility required for success in other species in relatively benign environments, one has to consider what chance vetiver has of producing a plant population from seed on (for example) the Byerstown Range with a fertility of 0.0025%, a tiny seed survival prospect, and a harsh and infertile environment for establishment.

A separate necessary consideration is the possibility of a change in circumstances leading to an increase in fertility of vetiver. One risk is of the future introduction of a fertile strain of vetiver. Such strains have been introduced to Australia and have been widely disseminated. They could be introduced privately as, for example, garden plants; or they could be substituted for Monto by unscrupulous vendors servicing public roadside stabilisation work. The obvious way of eliminating this latter risk is for MRD to ensure beyond doubt that only authentic material of Monto continues to be used in roadworks.

The danger of free-seeding strains cross-pollinating with existing Monto to allow it to produce fertile seed is apparently very low. There are numerous accounts overseas of infertile types of vetiver having been grown for very long periods in nurseries alongside fertile ones in conditions where cross-pollination has had every opportunity to occur, and yet of there being no drift in infertile stocks towards fertility.

Another risk to consider arises out of the possibility that Monto vetiver’s infertility is the result of a condition known as self-incompatibility, whereby the pollen of one plant cannot fertilise an ovule produced by that same plant. Self-incompatibility is common in many types of plant, including grasses. Monto vetiver, being a clone, may be derived from a single plant, and if it is, then from the point of view of self-incompatibility all plants are one and thus have an extremely low chance of fertilising one another. However, if a new plant arises from one of the occasional fertile seeds, it will be different genetically, a totally separate plant in its own right, and the barrier of self-incompatibility will no longer apply. Such a plant might then cross-pollinate successfully with either plants of Monto or of other seedlings, and produce seeds that would produce genetically diverse plant populations with no self-incompatibility, which might thenceforward be free-seeding. This possibility cannot entirely be discounted, as it is not yet known whether or not self-incompatibiltiy systems apply (There is active investigation of this possibility taking place at present in Taiwan). But in general it seems that the risk is very low, because, if it had been otherwise such change would have occurred and been noticed in the behaviour of plants grown in the nurseries mentioned earlier.

The balance of evidence, on present knowledge, thus leads inevitably to the view that the risk of Monto vetiver producing sufficient viable seed to place it in the category of a potential environmental weed is remote in the extreme.

Table 1. Records from which estimates of fertility of vetiver grass were made. Sampling dates, sampling sites, total numbers of loose spikelets recovered from samples, and numbers of caryopses found are tabulated.

Date	Site no.	Site locality	Number of spikelets	Number of caryopses
14/3/02				
	1	Byerstown Range (High)	13,200	0
	2	Byerstown Range (Laura R)	6,700	0
	3	Split Rock (North End)	6,900	0
	4	Split Rock (Turnoff)	13,500	1
	5	BlackMtns (Cktn Side)	11,800	0
	6	Trevethan Ck (N Side)	22,900	0
	7	Green Hills Cut (N Side)	18,300	0
	8	N of Archer Pt Turnoff	12,300	0
	Total for 15/3		105,600	1
28/3/02				
	1	Byerstown Range (Top)	19,000	0
	2	Byerstown Range (Laura R)	17,200	0
	3	Split Rock (North End)	8,500	0
	4	Split Rock (Art Site Turnoff)	10,000	0
	5	Black Mtns (Cktn Side)	19,300	0
	6	Trevethan Ck (NW of Creek)	21,100	1
	7	Green Hills Cut (Cktn Side)	20,100	1
	8	N of Archer Point Turnoff	16,800	0
	Total for 30/3		132,000	2
Grand total			237,600	3
Estimated number of sessile spikelets			118,800	
Ratio of sessile spikelets to caryopses			39,600:1	
% fertility of sessile spikelets			0.0025	

Total number of seed heads	About 615
Average number of sessile spikelets per head	193