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Swede associated toxicity in dairy cattle during winter 2014

An overview of the activities supported by DairyNZ

DairyNZ conclusions and recommendations for farmers

DairyNZ

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Disclaimer

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Funded by New Zealand dairy farmers through DairyNZ

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Executive Summary

The purpose of this document is to:

- Describe an unusual occurrence of illness and deaths in dairy cattle grazing swedes during the winter of 2014 in southern New Zealand (the 'Event')
- Present an overview of the results of the field and laboratory investigations that were conducted by DairyNZ
- Summarise the conclusions made by DairyNZ, particularly to avoid such losses in the future.

Most field work occurred in the period from September 2014 to February 2015. The laboratory and data analyses extended into mid-2015.

A multi-disciplinary group called the 'Swede Working Group' (SWG) was formed and this group, along with DairyNZ, assisted with the response to the problem.

Farmers surveyed

An initial rapid survey of dairy farmers in Southland and South Otago indicated that, on many farms, illness and death had occurred in animals grazing swedes.

In a later more comprehensive farmer survey, the incidence in pre-calving mobs (i.e. wintering of dry cows) was generally reported as 'modest'; i.e. median around 11 cases per 1,000 animals. During calving and early lactation, losses were significantly higher; i.e. median 34 per 1000 animals. However, in both circumstances a small number of farms had higher losses.

Cow losses across the Southland and South Otago regions during winter/spring 2014 cannot be estimated because a cross-sectional study was not completed. The number of cases reported here relate specifically to the comprehensive survey and cannot be extrapolated to estimate regional population loss.

Liver damage in cows

It was concluded that cow illness and deaths were the result of liver damage, possibly caused by higher levels of certain nitriles, as hypothesised by Collett et al. (2014)[1]. These compounds can be formed as breakdown products following the consumption and digestion of glucosinolates (GSL) found in all brassica species.

Of note, was evidence of liver damage found in apparently healthy animals grazing swedes in early spring 2014.

Information from the affected farms suggested that during this season, many cattle had been fed on swede crops that had moved into the 'reproductive phase'. There is reliable evidence in the scientific literature that this would result in exposure to higher concentrations of GSLs. A cross-sectional survey of swede crops in the Southland region aligned with this.

In the calving and early lactation period a strong statistical association was found between feeding the Herbicide Tolerant®' (HT®)[2] swede cultivar and illness or death of animals. Analysis of GSLs in swedes, found higher concentrations in upper leaves/stems and flowers in samples from HT® plants.

Conclusions

The key conclusions from the work undertaken by DairyNZ are as follows:

- All cultivars of swedes have the potential to cause liver damage in cattle and the impact, in terms of both morbidity and mortality, is a function of many factors i.e. a multi-factorial situation exists. These factors include;
  - the physiological status of the animal
  - plant characteristics (i.e maturity/cultivar/physical composition)
  - the effect of climate on plant characteristics
  - farm management practices.
- The higher disease incidence reported during calving and early lactation was strongly associated with increased exposure to swede crops in their 'reproductive phase'. As HT® swedes were shown to have higher concentrations of GSLs they exacerbated this risk.
- The air temperatures in the winter and spring of 2014 in Southland and South Otago were generally warmer than the 10 year average. In DairyNZ's opinion, these weather conditions contributed to swede crops retaining more leaf (which does not normally survive winter frosts) and the crops being in the 'reproductive phase' earlier than normal. This, therefore contributed to the clinical presentation of a known risk associated with feeding brassicas.

The findings of this work support the advice DairyNZ has provided to farmers over the last 15 months. This advice was aligned with managing the levels of GSLs consumed in the diet, particularly when swede plants begin the reproductive growth phase and GSL concentrations are expected to increase.

Recommendations

DairyNZ recommends that farmers:

- Do not feed HT® swedes on the milking platform in late August/early September (i.e. late pregnancy, early lactation). This is when many of the known factors (warmer temperatures, new leaf growth, 'bolting'/stem elongation) that lead to ill-heath and potential cow deaths can rapidly combine.
- Do not feed swede crops in their reproductive growth phase. This is recognisable when the swede's stem elongates, new growth appears and the swede plant develops flowers and a seed head (referred to as 'bolting').
- In autumn, before the first frosts, be cautious when grazing animals on swede crops as they might eat more leaves than bulbs as the bulbs are hard and difficult to eat.
- At any time during the season, be cautious when grazing animals on swede crops with a high leaf-to-bulb ratio, as cows may preferentially graze leaf.
- Observe the physical characteristics of the crop being fed, monitor the health of cows and adjust their feed management if incidences of ill-health are observed.
- Refer to DairyNZ Advisory #11 for more information around feeding management (Appendix 4).
- Follow PGG Wrightson Seeds endorsements (as at 30 November 2015) regarding HT® swedes and their use.

Table of Contents

1 Glossary

Item	Description
Attributable Fraction exposed (AF_e)	The proportion of disease in exposed herds that is due to the exposure
Attributable Fraction population (AF_p)	The proportion of disease in all herds that is attributable to the exposure
Bolting	A term used to describe the appearance of swede crops in the reproductive growth phase
Brassica species	A genus of plants in the mustard family (*Brassicaceae*). The genus is known for its important agricultural and horticultural crops and includes a number of weeds. It counts over 30 wild species and hybrids plus numerous cultivars and hybrids of cultivated origin. Swedes, along with many other crops, belong to the species *Br. napus.*
CMP or crop on milking platform	Crop fed on the milking platform during late pregnancy, calving and early lactation
Cumulative incidence	The proportion of non-disease individuals at the start that becomes diseased during the period of interest. It is a measure of the risk of an animal becoming affected.
Cumulative probability	A cumulative probability refers to the probability that the value of a data value falls within a specified range. All data (CP = 1.00 (or 100%)) is equal to or less than the maximum and no value (CP = 0.00 (or 0%) is less than the minimum). The median has a CP of 0.5 (or 50%); i.e. half the data is equal to or less than the median. A graph of the CP versus the data values in a sample or population is a useful way of presenting how data is distributed.
Cross-sectional study	A study in which disease and exposure statuses are measured simultaneously in a given population. This study type can be thought of as providing a 'snapshot' of the frequency and characteristics of a disease in a population at a particular point in time.
DairyNZ	The industry-good, levy-funded organisation that represents all New Zealand dairy farmers and invests in practical on-farm tools, science, resources and support and advocacy to ensure farmers have a profitable, sustainable and competitive future
Gamma glutamyl transferase or GGT	An enzyme found in many tissues, the most notable one being the liver. Increased levels of GGT are specific for damage to tissues associated with the bile ducts,
Glutamate dehydrogenase or GLDH	An enzyme found in many tissues in the body, including liver, kidney, intestine, muscle, and salivary gland. However, most of serum GLDH originates from liver cells both in health and disease states and thus it is a sensitive and specific marker of liver disease.
Glucosinolates or GSL	Natural components of many pungent plants including the brassicas. When plants are damaged or eaten glucosinolates are broken down by plant enzymes and by bacterial enzymes in the alimentary tract and toxic

Item	Description
	compounds can be formed.
Hepatotoxic	Substances that damage the liver
HT® swedes	A swede cultivar marketed by PGG Wrightson Seeds that is tolerant to the sulfonyl urea herbicide, DuPont® Telar®[1]
Incidence rate	The number of new cases of disease in a population per unit of animal-time at risk during a given time period. It is a measure of the rapidity with which new cases develop over time. In this report units are 'per 1000 animals per month'
Nephrotoxic	Substances that damage the kidney
Prospective study	A study that look forwards. May be experimental (subjects can be randomly allocated to a treatment/control group as in a clinical trial) or observational (the assignment of subjects into a treated group versus a control group is outside the control of the investigator).
P-value	The probability of getting the observed or more extreme results, assuming there is not an effect; i.e. the null hypothesis is true. When a hypothesis test is performed in statistics, a P-*value* helps determine the significance of the results.
Reproductive growth phase in swede crops or reproductive phase	The stage in the life cycle of swedes when the swede plant enters the reproductive stage; the stem of the swede elongates, new growth appears and the swede plant develops flowers and a seed head.
Retrospective study	A study that looks backwards in time. In a case/control study groups are selected according the presence or absence of a disease or condition. This type of study has the potential for confounding factors and bias selection to be present. The study is suitable for exploratory purposes and is often a precursor to a prospective study.
Risk factor	Any attribute, characteristic or exposure of an individual [or herd] that increases the likelihood of developing a disease or injury
Significance testing	The procedure of addressing the question: What is the probability that we think an effect or relationship between two variables is really just a chance occurrence? A probability of 5% or less (P value < 0.05) is commonly used to assign 'significance'; i.e. not just due to chance.
Wintering	The period between the end of lactation, when cows are non-lactating ('dry'), and while pregnant cows can still be transported before calving.
WMP	Wintering on the milking platform
WOF	Wintering off farm (i.e. away from the milking platform)

2 Introduction

During the winter and spring of 2014, Southland and South Otago dairy farmers and veterinarians[3, 4] observed unusual patterns of illness and death in cattle grazing on swede crops (the 'Event').

Sporadic reports were first received by veterinarians from mid-July to mid-August 2014. In mid-August a concerned veterinarian contacted other veterinarians seeking further information on such cases. A detailed chronology was described by Mark Bryan in a conference paper to veterinarians in June 2015[3]. In brief, he described:

- Sporadic reports and cases of dead and sick cows from mid-July to mid-August
- Comments from farmers that they had concerns with their swede crops
- Comments that the winter of 2014 had been particularly mild and that many swede crops had begun to flower
- That there was an unusual pattern of growth and maturity for swedes that was not typically seen until very late in the season (i.e. swede plants with elongated stems, seed heads forming or flowers)
- That famers were reporting palatability issues with swede crops; and
- That he was concerned about S-methyl-L-cysteine sulphoxide (SMCO) levels in swedes.

In mid-August 2014, DairyNZ, and other industry organisations, were alerted to the emerging issue. The general clinical picture, in addition to that identified above, was of ill-thrift, weight loss, and photosensitivity. Field staff reported that this syndrome continued through August but intensified as cows grazed crop on the milking platform during late pregnancy, calving and early lactation.

DairyNZ, along with a number of other organisations, responded to these events (Figure 1). An initial "advisory" to farmers was prepared and issued by DairyNZ on 5 September 2014. This advisory was also sent to rural professionals, New Zealand Veterinary Association (NZVA), Federated Farmers and Beef + Lamb New Zealand. A further 11 advisories, developed in collaboration with the SWG, were sent out between September 2014 and mid-October 2015.

A DairyNZ working group was formed in late August to manage DairyNZ's response activities. In early September, a multi-disciplinary team with a wide range of expertise in the areas of veterinary epidemiology, agronomy, scientific research, policy development, extension, animal husbandry, and communications called the 'Swede Working Group' (SWG) (Appendix 1) was established. At the first SWG meeting (17 September 2015) the SWG confirmed that an investigation into the Event be undertaken.

The intention of the activities was to understand the complex disease situation that was likely multifactorial in nature, involving; animal, plant, farm, farm management practices and, most likely, weather factors. The aims were to:

- Provide advice and support to impacted farmers to assist them in managing the immediate issues for the balance of the 2014/15 season
- Provide advice to manage the potential risks associated with feeding swede crops to dairy cows for 2015/16 and the future.

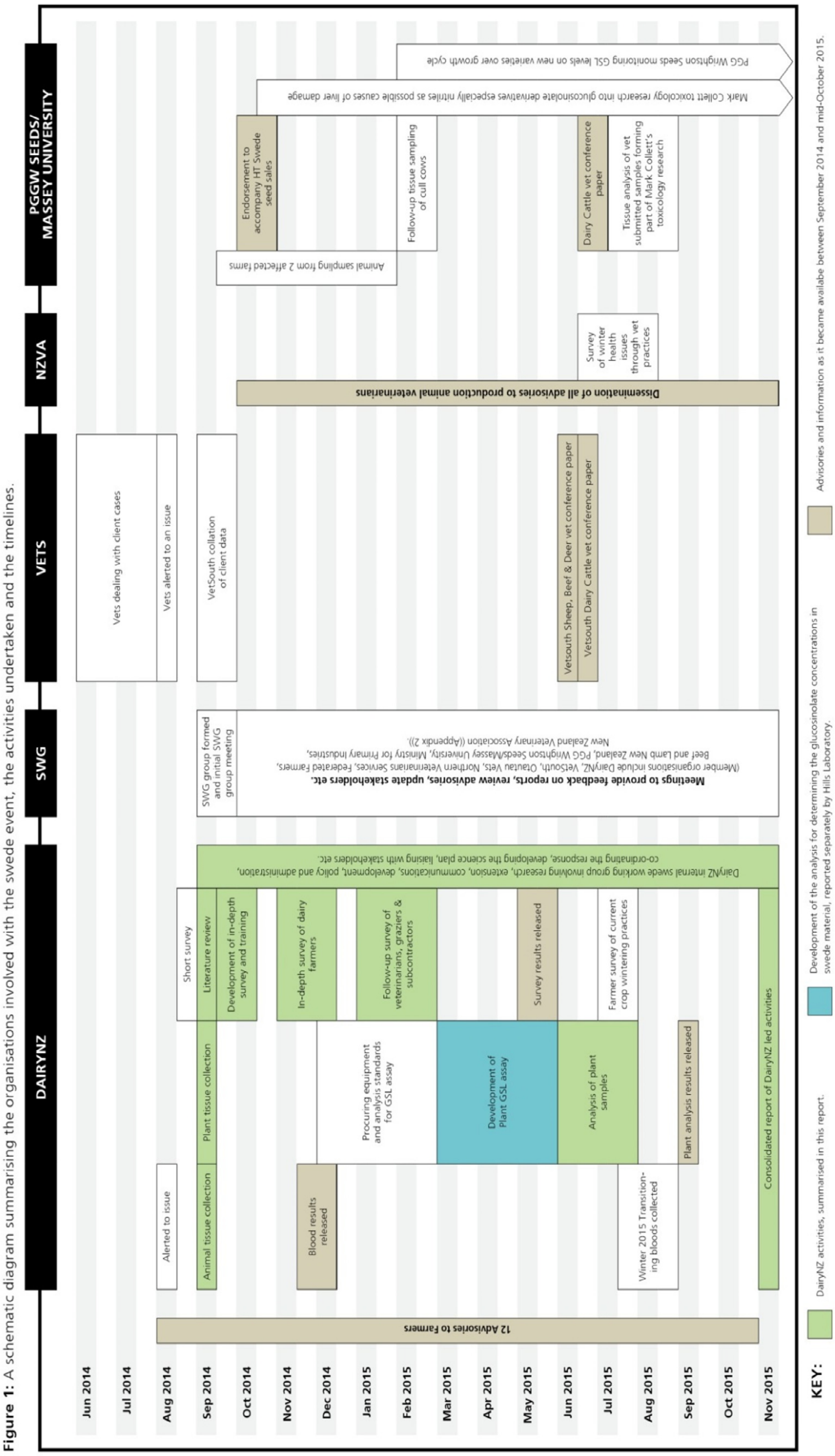


Figure 1 A schematic diagram summarising the organisations involved with the swede event, the activities undertaken and the timelines. The DairyNZ activities, summarised in this report are identified by the text boxes shaded green. The development of the analysis for determining the glucosinolate concentrations in swede material is reported separately by Hill Laboratories and is identified by the text box shaded blue. The text boxes that are shaded tan, relate to the advisories and information released as it became available between September 2014 and mid-October 2015.

3 Summary of DairyNZ activities

A timeline showing the work that was undertaken by DairyNZ and other groups is presented in Figure 1.

3.1 Initial activities

3.1.1 Advice and assistance for farmers

DairyNZ staff provided advice to farmers to assist them with managing affected animals and disposing of residual plant material.

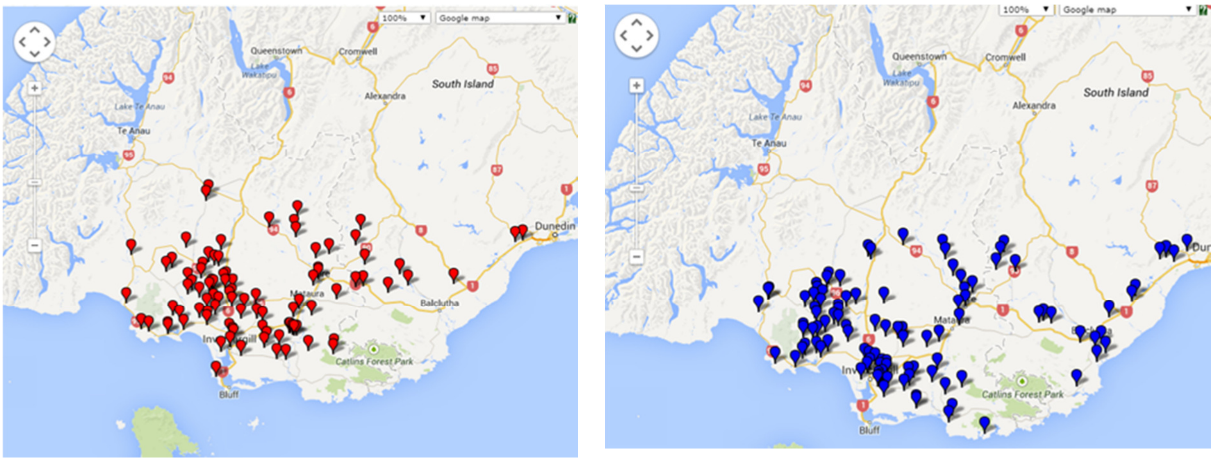
3.1.2 Collection of plant and animal samples

Priority was given to collecting animal samples to confirm clinical diagnoses, and to obtain representative samples of swede crops for analyses.

3.1.3 A rapid survey

A rapid survey was undertaken, unrelated to the collection of plant and animal samples, to assess how many properties were affected. Approximately 2,600 farmers representing 1300 farms in Southland and South Otago were contacted by email. Of the total 406 respondents, 313 (77%) replied that they had fed swedes to dairy cows during preceding months. Of these, 132 (42%) indicated that cows had experienced greater than normal health problems while 58% indicated their experience had been as expected (Figure 2). Affected farms were spread across the region.

Figure 2 Spot maps, obtained from the rapid survey indicating the location of farms that responded. Left: farms where illness/deaths in cattle grazing swedes were reported. Right: farms where no animal health issues were reported.



3.2 Consolidation and analysis

3.2.1 Literature review

An extensive review of the scientific literature was undertaken to:

- Ensure the working team were up to date with potential issues associated with grazing brassica crops and, in particular, swedes
- Identify potential causes and to help guide the development of the in-depth survey.

3.2.2 An in-depth survey

An in-depth survey was designed, implemented and analysed. This included questions specifically addressing known and potential causal factors of brassica toxicity.

3.2.3 Analysis of animal and swede samples

Analysis of the animal samples was completed using the commercially available testing service offered by Gribbles, Invermay.

Analysis of the swede material was more challenging, given that there were no agreed laboratory protocols for testing GSLs in swede material. Additionally, there were no accredited laboratories undertaking GSL testing in New Zealand. Options for completing the analysis overseas were considered. However, DairyNZ considered an important outcome should be to have, available in New Zealand, a validated method for testing individual GSL and SMCO concentrations by an accredited commercial laboratory. This was also supported by the SWG. Plant analysis was consequently delayed while a commercial laboratory was selected, contracted, the equipment procured from overseas and the analytical procedure developed.

3.3 Dissemination of findings and recommendations

Communications was the key activity throughout the project and has occurred in parallel with other activities. During this phase, advisories detailing best-practice crop and animal management were issued, to help manage the risks associated with feeding swede crops to dairy cows during the 2015/16 season. New information from the specific swede activities described previously was released to farmers and their advisers (veterinarians and other rural professionals), and the media, as it became available.

This report is the final part of the project.

4 *Brassica* species and animal toxicity

4.1 Background

Brassica crops (in particular rape, kale turnips and swedes) are an essential source of supplementary feed on Southland and South Otago farms. Their reliable fast-growing characteristics mean that high-quality readily digestible feed can be produced within a wide range of climatic conditions especially in Southland and South Otago where they are used to fill feed deficits. Swedes in particular have been fed to dairy cows in Southland and South Otago for over 50 years.

However, it has been well documented that a number of animal health problems are associated with the ingestion of brassica crops.

Given the reports of illness and death in dairy cattle being fed swedes, DairyNZ considered that an up-to-date statement of current knowledge was an important component of the response. To this end, a review of the scientific literature was commissioned. This report presents a synopsis of the full review and includes the references sources in Section 4.2.3.

4.2 Key outcomes of the scientific review

4.2.1 Overview

It is well established that the *Brassica* species are rich in sulphur-containing amino acids and other sulphur-containing substances such as GSLs, which occur in all economically important varieties of *Brassica*. Many are 'pre-toxins', meaning that they can break down into substances that are toxic for animals when plant tissue is damaged (as occurs during ingestion and digestion).

These substances, often with bitter and unpalatable tastes, have evolved by the plant as protective mechanisms against disease, insect attack and herbivore infestation. Concentrations generally increase as the plant reaches maturity; flower-heads and seed tend to have the highest concentrations. The diversity of these substances and their break-down products result in a spectrum of brassica-associated diseases, which present with a wide range of symptoms.

The commonly recognised clinical syndromes associated with ingestion of brassica crops are listed in Table 1.

Table 1 Animal health diseases associated with the ingestion of brassica crops

Clinical syndrome	Pathogenesis
Haemolytic anaemia	The 'pre-toxin' S-methyl-L-cysteine sulphoxide (SMCO) is converted into dimethyl disulphide by rumen microflora. This 'toxin' causes oxidation of haemoglobin in red blood cells with Heinz body formation and haemolysis in the spleen.
Turnip photosensitisation (hepatogenous photosensitisation)	Symptoms develop secondary to damage to bile ducts that is induced by an unknown toxin similar, in many respects, to that induced by sporidesmin (which results in facial eczema). The subsequent photosensitisation occurs through the same pathway.

Clinical syndrome	Pathogenesis
Thyroid disease and induced iodine deficiency	Associated with very high levels of GSLs in feeds made from brassica crops.
Nitrate poisoning	Associated with feeds containing high levels of nitrates. The rumen microflora convert nitrate to nitrite, which, if absorbed into the blood, can oxidize haemoglobin, thus interfering with oxygen transport.
Rape blindness	May occur as a consequence of the high quantities of sulphur found in *Brassica* spp. When released in the rumen, sulphur destroys thiamine resulting in thiamine deficiency which can lead to brain lesions (i.e. polioencephalomalacia).
Acute pulmonary oedema and emphysema	Reported when hungry cattle get sudden access to brassica crops containing high quantities of the amino acid L-tryptophan. In the rumen 3-methyl-indole is produced from L-tryptophan. This chemical induces toxic injury in the lungs.
Rape scald (photosensitisation)	Reported in lambs grazing immature rape crops. Pathogenesis is unknown.
Rickets	Brassica crops have low phosphate levels which may lead to rickets, especially in young growing animals.
Induced selenium and copper deficiency	The high sulphur content can interfere with uptake of selenium and copper when fed for a prolonged period.

4.2.2 Active compounds found in *Brassica* species

The literature suggests that there are two broad classes of toxic compounds that can be produced. One group damages red blood cells and can result in anaemia. The other group, derivatives of the GSLs, contains substances that can interfere with thyroid function or damage the liver and kidney.

4.2.2.1 Derivatives of S-methyl-L-cysteine sulphoxide (SMCO)

SMCO is converted into dimethyl disulphide by rumen microflora. This 'toxin' causes oxidation of haemoglobin in red blood cells with Heinz body formation and, thus, a higher rate of haemolysis in the spleen. Excessive destruction of red blood cells can lead to anaemia. Haemoglobin can spill over into the urine and thus 'red water' is a common symptom.

4.2.2.2 Hydrolysis products of glucosinolates (GSL)

A glycoside is a molecule in which a sugar is bound to another functional group by a glycosidic bond.

Glucosinolates are sulphur-containing glycosides. The sugar thioglucose is bound to a sulphur containing side-chain which varies according to the amino acid from which it was derived. Nearly 130 unique GSL's have been described and classified according to their derivative amino acid structure.

The most commonly identified GSL in brassicas are listed in Table 2.

Table 2 Common and chemical names of the most commonly identified glucosinolates in *Brassica* species and their categorization according to their amino acid side-chain moiety

Common name	Chemical name	Category
glucobrassicin	indol-3-ylmethyl GSL	Indolic (tryptophan)
glucobrassicanapin	4-pentenyl GSL	Aliphatic; alkenyl subgroup
gluconapin	3-butenyl GSL	Aliphatic; alkenyl subgroup
gluconasturtiin	2-phenylethyl GSL	Aromatic (tyrosine, phenylalanine)
neoglucobrassican	1-methoxyglucobrassican	Indolic (tryptophan)
progoitrin (glucorapiferin)	2-OH-3-butenyl GSL	Aliphatic; alkenyl subgroup
sinigrin	2-propenyl GSL; allyl GSL	Aliphatic (methionine, alanine, valine, leucine, isoleucine)

Notes:

- Intact GSLs are stable and non-toxic
- When plant cells are crushed, as occurs during ingestion, the plant's myrosinase enzyme system is activated to hydrolyse the GSLs
- Hydrolysis yields glucose, acid sulphate ions, ascorbigens and unstable intermediate products that rapidly undergo molecular rearrangement to create diverse products that are potentially toxic if ingested in sufficient amounts
- Potentially toxic compounds include the thiocyanates, isothiocyanates, oxazolidine-2-thiones, nitriles, and epithionitriles.

4.2.3 Glucosinolate levels in brassica forage crops

In the review, literature was cited attesting to:

- Limited effects of phosphate and potassium fertiliser on GSL levels in rape and turnip crops
- In rape much of the variations in GSL content of both leaf and stem could be explained by plant genotype, but the stage of maturity, the date of sowing, and the plant part (leaf or stem) also influenced concentrations
- Limited effects of sulphur and nitrogen fertiliser on GSL levels in kale.

The published literature indicates that while GSL levels may vary with plant genotypes, between parts of the plant, and with the plant's stage of maturity, other factors such as soil type, fertiliser management and climatic conditions can also cause GSL content to vary. Furthermore, there are many factors that influence the direction of rumen hydrolysis into the various breakdown products, as well as inherent variations between these products in their stability, longevity and toxicity.

This makes it difficult to identify specific toxins that cause liver and kidney damage, and accordingly their parent GSLs. The general approach therefore when assessing GSL levels in crops is to examine both total GSL content, and the proportions of those GSL that predominate.

4.2.4 Toxicity of glucosinolate derivatives in fodder crops

While generally resulting in poor growth rates, in young stock, the primary toxic effects of GSL derivatives have two main effects:

- Interference with thyroid function as a result of exposure to the thiocyanate, isothiocyanate and oxazolidine-2-thione derivatives
- Liver (bile duct hyperplasia and liver necrosis) and kidney dysfunction as a result of exposure to the nitriles and epithionitriles derivatives.

A review by Tripathi and Mishra (2007) summarises the biological effects of GSL on animals. They reported:

- Ruminants are comparatively more tolerant to GSL intake than other species and adults are more tolerant than young animals
- A dietary GSL concentration of 11 µmol/g dry matter in the diet should be safe
- between 11.7 and 24.3 µmol/g dry matter in the diet resulted in reduced feed intake and milk production in dairy cows
- Concentrations above 31.0 µmol/g dry matter, amounting to a daily intake of 44 mmol GSL per day per cow caused thyroid disturbance and depressed cow fertility.

There is little published literature about levels of individual GSL derivatives at which toxicity is induced in humans and rodents. Toxicity data in ruminants are even sparser. The literature indicates that adverse effects in animals have generally been correlated to the amount of total GSLs in the diet, despite toxicity of individual GSLs derivatives being unknown.

4.3 Source material

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5 Field observations: animal health

5.1 The initial field investigation

5.1.1 Background

In the initial response phase, regional DairyNZ staff worked with farmers and veterinarians to collect crop and animal samples from farms where there were affected animals and from a convenient sample of farms where nothing unusual had been observed (control farms). These farms were known to DairyNZ or recommended by their veterinarian and were willing to participate. Crop sampling was paired with animal sampling. Even at this early stage, there were questions about the role a recently introduced swede type: Herbicide Tolerant® (HT®). Therefore, farms with and without HT® crops were purposively (i.e. non-randomly) selected.

Animal samples were collected from 12 farms where a range of swede varieties were being fed. Blood samples were taken from a total of 121 animals, including from two severely affected cows from which samples were taken prior to euthanasia and necropsy (Table 3).

Table 3 Summary of the blood samples collected from dairy cattle being fed swedes in September 2014 during the swede associated liver disease event in Southland and South Otago.

<table>
<tr><th>Herd Affected*</th><th>Number herds</th><th>Clinical cows sampled</th><th>Number animals</th><th>Severity</th><th>Number animals</th></tr>
<tr><td rowspan="3">Yes</td><td rowspan="3">4</td><td rowspan="2">Yes</td><td rowspan="2">10</td><td>High</td><td>2</td></tr>
<tr><td>Low</td><td>8</td></tr>
<tr><td>No</td><td>30</td><td>-</td><td>30</td></tr>
<tr><td>No</td><td>8</td><td>-</td><td>-</td><td>-</td><td>81</td></tr>
<tr><td>Total</td><td>12</td><td>-</td><td>-</td><td>-</td><td>121</td></tr>
</table>

* Farmer perception (verbal or typical cases of illness or deaths associated with feeding swedes)

Preserved tissues from the two animals necropsied were submitted to a veterinary pathology laboratory for histological examination. All blood samples were submitted for a range of assays. These covered a wide range of disease processes, as follows:

- liver damage
- muscle damage
- energy imbalance
- mineral and electrolyte imbalance
- protein metabolism imbalance
- inflammation
- anaemia

5.1.2 Key findings

5.1.2.1 Necropsy results

Prior to euthanasia, both cows were subject to a full clinical examination. Both exhibited unusual nervous signs and one was apparently blind in one eye. One showed evidence of photosensitivity; i.e. ruptured blisters on the teats.

The carcases of both were severely jaundiced. The livers were much enlarged with rounded edges.

The pathologist reported that one animal exhibited 'chronic fibrosing lymphocytic cholangitis', which was typical of that seen in swede toxicity'. Unfortunately a sample of liver from the other cow was not available for examination.

5.1.2.2 Blood test results

As might be expected, the blood test results from animals suffering sub-acute or chronic disease are complex as secondary disease processes may be present. However, a notable observation in many cases was elevated indicators of liver disease; in particular gamma glutamyl transferase (GGT) and glutamate dehydrogenase (GLDH). Both of the animals that were necropsied had elevated levels as did many of the 10 other clinically affected cows from other farms.

The log transformed[c] individual animal GGT and GLDH assays are presented in the Figures 3 to 6. Some notable observations are as follows:

- Many animals from both properties where cases (illness/death) had occurred (case farms) and had not been observed (control farms) had elevated GGT levels. Fifty percent (50%, 59/119) of all the animals sampled had levels that exceeded the quoted 'normal upper limit' (37 IU/l or $\log_{10}$ = 1.5682) (Figure 3). There are no statistically significant associations[d], in terms of 'negative 'and 'positive 'animals, (P > 0.05) between cases and control farms, nor between HT® and non-HT® farms (Figure 4).
- The GLDH results were more contrasting on both case and control farms, and on HT® and non-HT® farms (Figures 5 and 6). In the former, 34% (13/38) of animals on case farms had elevated levels (i.e. > 59 IU/l or $\log_{10}$ = 1.7708) versus 19% (15/81) in the control farms (P = 0.068). In the latter (HT® or non-HT®), of HT® farms 28% (25/89) had elevated levels versus 10% (3/30) (P = 0.049).

[c] The assay results were highly right skewed; by taking the log this was "transformed" into a normal distribution which is more suitable for analysis.
[d] These analyses (i.e. the χ^2 test) assume independence; i.e. that the animals are grouped in herds is ignored. However, with respect to achieving statistical significance, this is the most likely scenario. This was confirmed using multi-level mixed effect modelling with herd as a random effect; all P-values were shifted towards 1.00; i.e. all P-values are > 0.05.

Figure 3 Individual cow gamma glutamyl transferase (GGT) results from blood samples collected from affected (case) and non-affected (control) farms in Southland during spring 2014. Units are log_{10} (international units/litre). The normal upper limit (1.5682) is also shown.

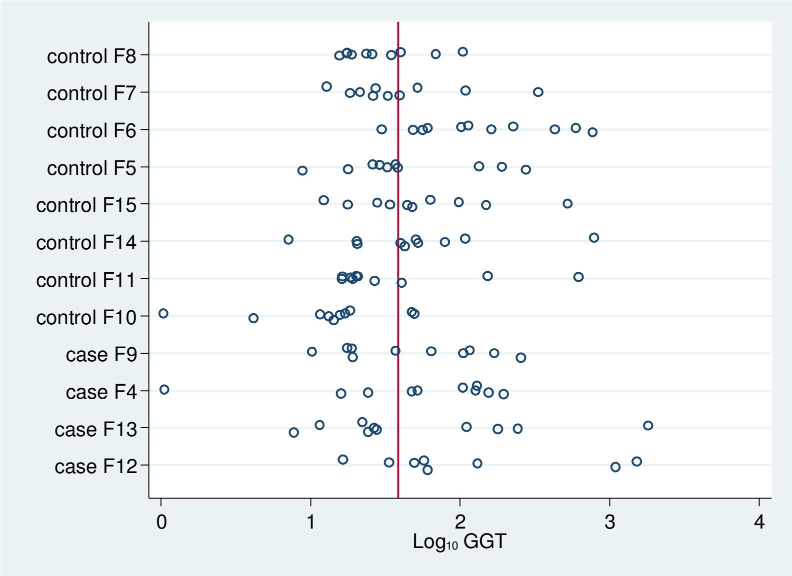


Figure 4 Individual cow gamma glutamyl transferase (GGT) results from blood samples collected from farms with HT® and non-HT® swedes in Southland during spring 2014. Units are log_{10} (international units/litre). The normal upper limit (1.5682) is also shown.

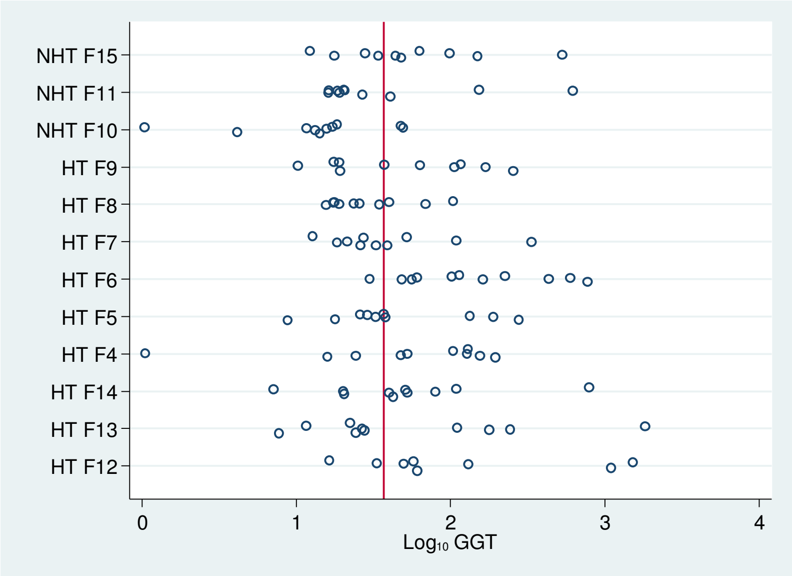
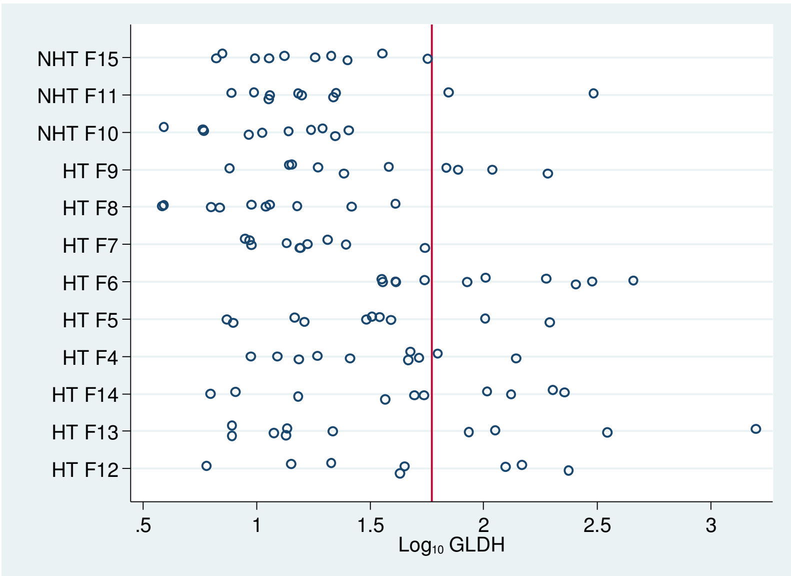


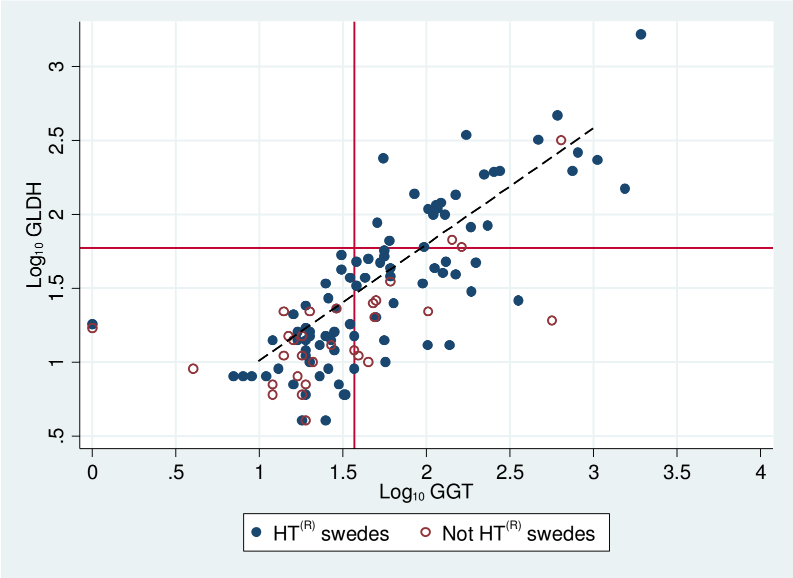
Figure 5 Individual cow glutamate dehydrogenase (GLDH) from blood samples collected from affected (case) and non-affected (control) farms in Southland during spring 2014. Units are $\log_{10}$ (international units/litre). The normal upper limit (1.7708) is also shown.

Figure 6 Individual cow glutamate dehydrogenase (GLDH) results from blood samples collected from farms with HT® and non-HT® swedes in Southland during spring 2014. Units are $\log_{10}$ (international units/litre).The normal upper limit (1.7708) is also shown.



In Figure 7, the GGT and GLDH results for each animal are plotted on a scattergram, with the HT® status shown in either solid blue circles (+ HT®) or open red circles (- HT®). A line representing the overall ratio of GLDH/GGT (i.e. 0.85 to 1) is also presented. The two assay results are strongly correlated (r = 0.76, P < 0.001). The data also indicate that 'GGT–positive'[e] animals grazed on HT® swedes are more likely to be 'GLDH-positive' (i.e. 52% (25/48) +HT® versus 27% (3/11) -HT®); however, this does not achieve statistical significance (P = 0.187, Fisher's exact test).

Figure 7 Scattergram of $\log_{10}$ transformed GGT versus GLDH blood assays for each cow sampled during the swede associated liver disease event in Southland during spring 2014. Farm status in terms of HT® swedes (blue solid circle) or not (red open circle) is shown. The normal upper limits for GGT (1.5682) and GLDH (1.7708) are shown. The overall ratio of GLDH/GGT is also shown (black dashed line).



The veterinarian supervising this work noted that 'liver damage from sporidesmin poisoning (facial eczema) follows similar pathology associated with bile duct damage[5]. In facial eczema cases, the absolute levels of liver damage enzymes measured are not well correlated to actual level of damage or to prognosis for recovery[6]. Clearance rates for liver enzymes are generally in the order of several weeks[7]. Sources of variation in a group of cows sampled at a single point in time include both the severity of a toxic incident, and the time since this incidence occurred. Low levels can therefore reflect recent mild damage or recovery from previous more severe damage.

The project team concluded that 'There is evidence of subclinical disease with all swede varieties grazed during this period'.

The results support the view that this disease is due to bile duct damage, since GGT is secreted at higher levels from the bile duct linings when this occurs. While there is an apparent trend for more animals to have blood parameters suggesting subclinical disease on farms fed HT® swedes, this is a limited dataset and any inferences drawn should be viewed with caution.

The screens for other putative disease processes yielded little, as detailed in Table 4.

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[e] i.e. greater than the normal upper limit

It was concluded that 'There is evidence of subclinical disease with all swede varieties grazed during this period'.

Table 4 Summary of results of other blood screens for putative disease processes determined from blood samples collected from affected and non-affected farms in Southland during the swede associated liver disease outbreak in spring 2014.

Disease Process	Comment
Muscle damage	One of the animals necropsied had elevated creatinine and urea most likely a result of dehydration or renal failure. No other significant findings.
Energy imbalance	Indicators generally within the reference range for cows entering the calving transition period.
Minerals and electrolytes imbalance	Magnesium, calcium, sodium, chloride, potassium and bicarbonate within normal parameters on all 11 farms. Serum phosphate levels were low on two farms but not related to toxicity issue.
Protein metabolism	Indicators suggest that protein nutrition on all farms was generally adequate.
Inflammation	Changes in the inflammatory proteins were observed on all farms, irrespective of the swede variety grazed, suggesting chronic low-grade inflammation in about 50% of cows. This may represent more generalised consequences of winter management systems for dry dairy cows that include grazing swedes. Mean WBC, neutrophil and eosinophil counts were within the normal reference ranges on all farms.
Anaemia	With exception of one farm, mean red blood counts were within the reference range on all farms. However, on some farms the prevalence of anaemia was sporadic to moderate. It was suggested that there is a case for determining SMCO concentrations in plant material to confirm that this is not in sufficient concentrations to cause toxic effects (see Table 18).

5.2 Interviews with owners or managers of affected herds

5.2.1 Background

Following the initial assessment of the problem, work immediately started on an in-depth survey of putative risk factors associated with the disease outbreak. In this 'retrospective' study a total of 134 owners/managers of a sample of herds, both affected and not affected, and 34 graziers were interviewed.

The respondents from the rapid survey provided the basis for selection of candidates for the in-depth survey. Respondents were listed in random order within group and contacted sequentially to ascertain their agreement to participate in the survey. Where respondents were not available for an interview, or could not be contacted after several attempts, they were removed from the list. The status of these herds (i.e. either 'case' or 'control') was resolved during a period of consultation with veterinary practitioners (see Figure 19).

This survey was undertaken between November 2014 and February 2015 and the results are reported in Section 6. In these interviews information and data concerning the nature of the disease in affected herds was also collected.

Data were available from a total of 78 herds where dairy cattle had grazed swedes and there had been illness or deaths attributed to these crops. Herd owners/managers were offered a list of disease syndromes, some quite general (e.g. unexplained weight loss) and others very specific (e.g. goitre). In addition, they were requested to indicate the stock classes which had been affected. The stock classes were:

- Yearlings (i.e. rising one year olds)
- Heifers (i.e. rising two year olds)
- Dry cows, mixed age greater than two years old
- Milkers, mixed age greater than two years old

This data were simply 'presence/absence' within a stock class for each affected herds.

Part of the interview included collecting very detailed data on supplementary feeding that had taken place both prior to calving (i.e. 'wintering') and during calving/early lactation. With respect to the former, this was also divided into 'wintering off farm' and 'wintering on the milking platform'. Thus, there were three scenarios separated in either time or location:

- Wintering off farm (WOF)
- Wintering on the milking platform (WMP)
- Swede crop fed during calving and early lactation (CMP)

In all herds some/all of the animals had been fed swedes during at least one of these scenarios. However, the three scenarios might not have been applicable to all herds. Further, illness and deaths may or may not have occurred during a particular scenario. It was necessary to take account of this somewhat complex picture when undertaking the analysis of these animal health data associated with supplementary feeding.

5.2.2 Key outcomes

5.2.2.1 Clinical symptoms

The initial analysis focussed on the question 'what symptoms or disease syndromes were seen in the affected herds?' (Figure 8). Issues associated with body condition, sudden death and photosensitivity were seen in around two-thirds of herds, with metabolic diseases and reproductive problems in around half.

In Figure 9, the syndrome 'reduced body condition' is broken down. 'Unexplained weight loss' is prominent.

In Figure 10, 'reproductive problems' is likewise broken down. This suggests that calving problems, retained membranes and metritis were all issues in around 40% of herds.

Figure 8 Disease syndromes reported in the affected herds in cattle grazing swedes during the swede associated liver disease event in Southland in winter/spring 2014. Each bar represents the percent of herds exhibiting each syndrome. The actual percentage is also adjacent to the bar.

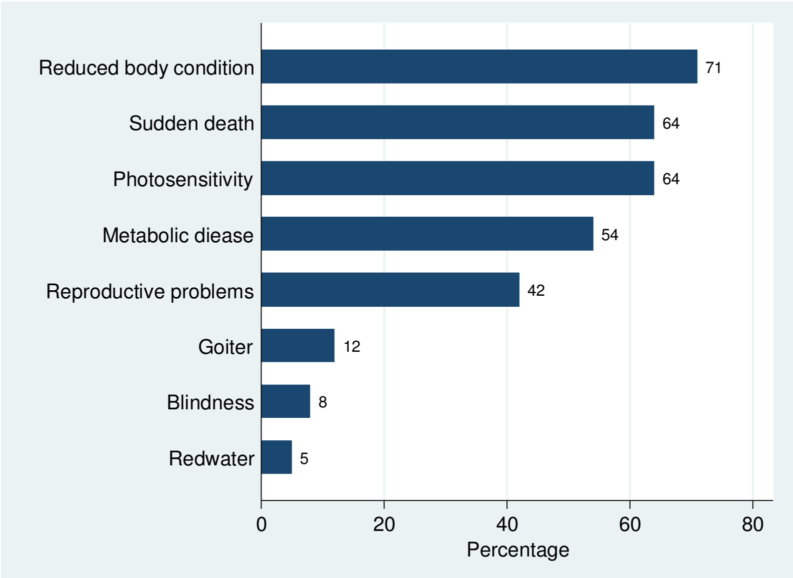


Figure 9 Body condition' syndromes reported in the affected herds in cattle grazing swedes during the swede associated liver disease event in Southland in winter/spring 2014. Each bar represents the percent of herds exhibiting each syndrome. The actual percentage is also adjacent to the bar.

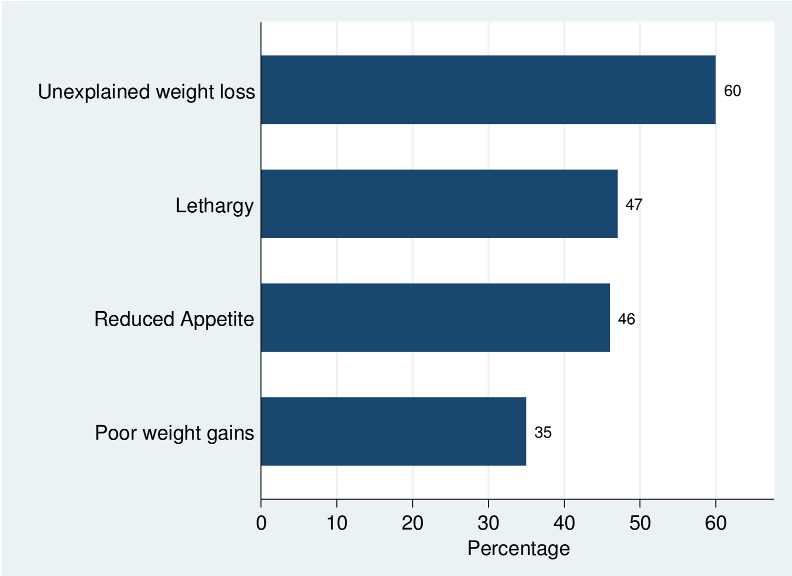
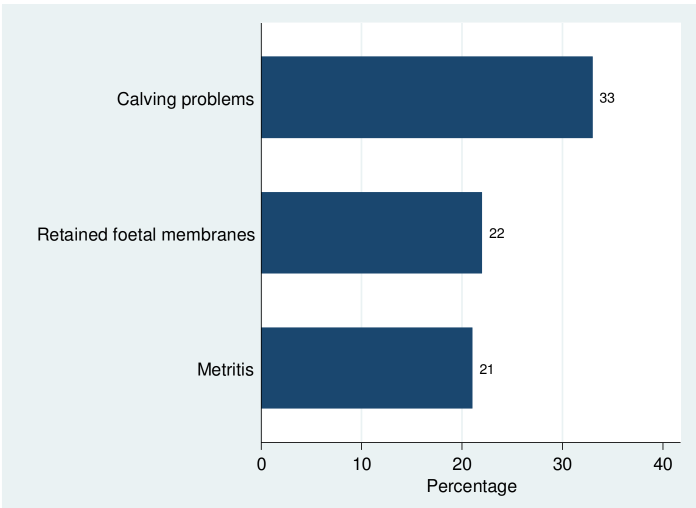


Figure 10 'Reproductive problems' reported in the affected herds in cattle grazing swedes during the swede associated liver disease event in Southland in winter/spring 2014. Each bar represents the percent of herds exhibiting each problem. The actual percentage is also adjacent to the bar.



The nature of the data (i.e. presence/absence) present some difficulties when investigating the symptoms reported in the various stock classes. A relevant question is 'are there marked differences between the classes?'.

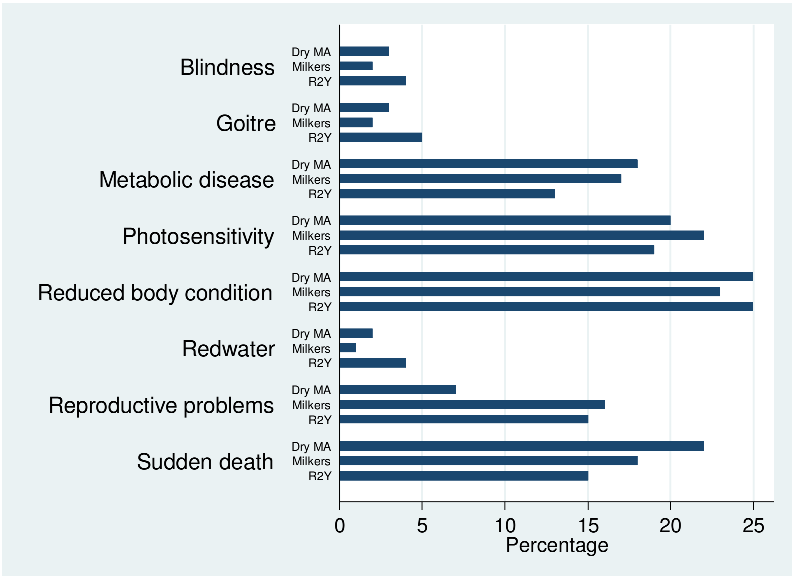
An insight into the relative occurrence of the reported syndrome observed in different stock classes was achieved by summing the incidences of reported syndromes observed in each herd by stock class and then calculating the proportion of affected herds in each of the named syndromes. The results of this are presented in Table 5 and Figure 11.

The data relating to rising one year olds were too sparse to be meaningful. For the other classes, it was remarkably consistent across all three. In the case of 'reproductive problems' the figure for dry cows (7%, 11/159) is around half that for milkers and rising two year olds. However, this may have been the result of disease well before calving.

Table 5 Relative occurrence of disease syndromes reported in rising one year olds, rising two year olds, mixed aged dry cows and milkers at the herd level during the swede associated liver disease event in Southland in winter/spring 2014.

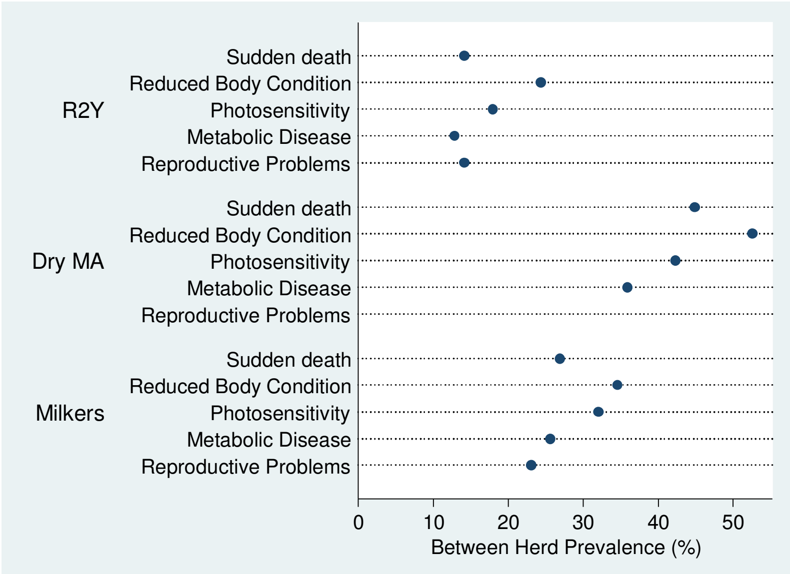
Syndrome	Rising one year olds	Rising two year olds	Mixed aged dry cows	Milkers
Sudden death	25% (3/12)	15% (11/75)	22% (35/159)	18% (21/116)
Reduced body condition	58% (7/12)	25% (19/75)	25% (41/159)	23% (27/116)
Photosensitivity	17%(2/12)	19% (14/75)	20% (33/159)	22% (25/116)
Metabolic disease	N/A	13% (10/75)	18% (28/159)	17% (20/116)
Red water	0	4% (3/75)	2% (3/159)	1% (1/116)
Blindness	0	4% (3/75)	3% (4/159)	2% (2/116)
Goitre	0	5% (4/75)	3% (4/159)	2% (2/116)
Reproductive problems	N/A	15% (11/75)	7% (11/159)	16% (18/116)

Figure 11 Disease syndromes reported in rising two year olds (R2Y), dry mixed age cows (Dry MA) and milkers during the swede associated liver disease event in Southland in winter/spring 2014. Each bar represents the percent of total reported syndromes that each disease syndrome constituted.



An alternative approach was to calculate the proportion of herds in which a syndrome had been observed, for each stock class. The results of this are illustrated in Figure 12. This indicates (assuming equal frequency of observation of stock classes), as above, that the relative occurrence of each major syndrome in each stock was similar, but that 'disease or death' was reported more commonly in dry mixed age cows than milkers and rising two year old animals. This probably reflects exposure of each stock class to swedes.

Figure 12 Disease syndromes reported in rising two year olds (R2Y), dry mixed age cows (Dry MA) and milkers during the swede associated liver disease event in Southland in winter/spring 2014. Each point shows the proportion (%) of all herds in which each disease syndrome was observed in each stock class.



Generalised 'illness' and 'death' were prominent features and it is of note that in each herd the numbers 'ill' and 'dead' were moderately correlated ($r = 0.54$, $P < 0.001$) but there were some outliers. In two herds despite many animals being ill, compared with other herds the number of deaths was relatively modest. If the data from these herds is excluded, the correlation coefficient (i.e. between 'illness' and 'death') rises to 0.65; a strong correlation. This data gives some insight into how serious physiologically this condition was; i.e. a high case fatality of around 50%.

5.2.2.2 Disease incidence

In epidemiology, the term 'incidence' refers, in general, to the number of new cases of a disease or condition, but in scientific investigations two specific measures are commonly used. The first of these is 'cumulative incidence' (CI), which is the proportion of non-disease individuals at the start that become diseased during the period of interest (May to September 2014). It is a measure of the 'risk' of an animal becoming diseased, dying, etc. The second is the 'incidence rate' (IR), which measures the rapidity with which new cases of a disease develop over time.

As noted above, data concerning the total number of animals ill or dead for three scenarios were collected, via farmer recall or written records from the farm or veterinary practice, during the intensive survey, as follows:

- During wintering off the milking platform (WOF)
- During wintering on the milking platform (WMP)
- Swede crop being fed during calving and in early lactation (CMP).

The CI[f] reported for each group[g] in each of these scenarios is right skewed, especially for WOF and CMP (i.e. many lower figures relative to the number of higher values). This is demonstrated in Figure 13. In such circumstances, the average is highly influenced by the small number of high values and thus the median is a better indicator of the population status (Table 6).

Figure 13 Cumulative incidence (CI) per 1000 animals for three wintering scenarios (wintering off farm, WOF; wintering on milking platform, WMP; and swede crops fed during calving and early lactation, CMP) identified during the swede associated liver disease event in Southland in winter/spring 2014. Graphs show the cumulative probability[h] versus the cumulative incidence values.

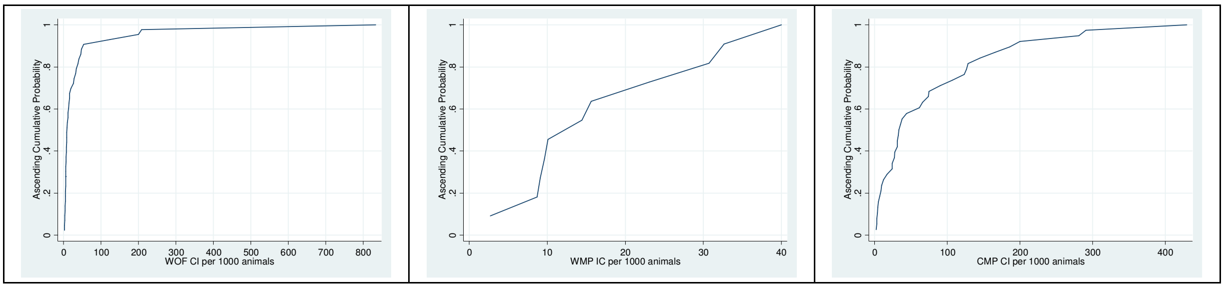


Table 6 Cumulative incidence (CI) per 1000 animals for three wintering scenarios (wintering off farm, WOF; wintering on milking platform, WMP; and swede crops fed during calving and early lactation, CMP) during the swede associated liver disease event in Southland in winter/spring 2014. Table shows number of different groups (n), median number of animals affected and 95% confidence limits (CL) of the median.

Scenario	n	Median	Low 95 CL	Upper 95 CL
WOF (Wintering off farm)	43	9	6	16
WMP (Wintering on milking platform)	11	14	9	31
CMP (Crop fed calving and early lactation)	38	34	25	74

Using log transformed CI data, an ANOVA was conducted, followed by a pairwise comparison using the t-test. The ANOVA returned a highly significant P-value (P = 0.004); CMP versus WOF also returned a significant difference (P = 0.004), but CMP versus WMP and WOF versus WMP returned P-values of 0.133 and 1.000 respectively. It should be noted that, the number of groups in the WMP is low (n = 11).

The time spent on swede and other crops during the wintering period (i.e. pre-calving) was around double that under the CMP scenario (Table 7; i.e. two months versus one month).

[f] ((Number ill + Number dead)/(Number on Crop)*1000)
[g] Either the whole herd or part of a herd
[h] See glossary

Table 7 Period (days) spent on crops during wintering off farm (WOF), wintering on the milking platform (WMP) and during calving/early lactation (CMP) for surveyed farms in Southland and south Otago during winter/spring 2014. Average with 95% confidence limits (CL), minimum and maximum values.

Scenario	Average	Lower 95% CL	Upper 95% CL	Minimum	Maximum
WOF	60	55	67	8	123
WMP	64	51	77	28	85
CMP	30	26	34	13	67

In Figures 14 to 16, the time spent on the crop and the cumulative incidences for each scenario are plotted in scattergrams. Whether or not HT® swedes were the crop is also shown. Points of interest are as follows:

- CI and time on the crop are generally poorly correlated (r = -0.10 P = 0.544, r = -0.35 P = 0.290 and r = 0.045 P = 0.788, for WOF, WMP and CMP respectively)
- As noted above, the longer period on the crop pre-calving
- All but two of the CMP groups were on HT® swede crops
- The CI's seen with HT® swedes and other crops are across a similar range.

Only in the WOF scenario is there sufficient data to conduct a statistical analysis of effect of the factor 'HT® swedes' on CI. This is not significant (P = 0.201).

Figure 14 Wintering off farm (WOF) $\log_{10}$ cumulative incidence (per 1000 animals) versus days on crop for HT® swedes (blue closed circles) and other swede varieties and crops (red open circles) summarised from data collected during a survey of dairy farmer wintering practices in Southland and south Otago during winter/spring 2014.

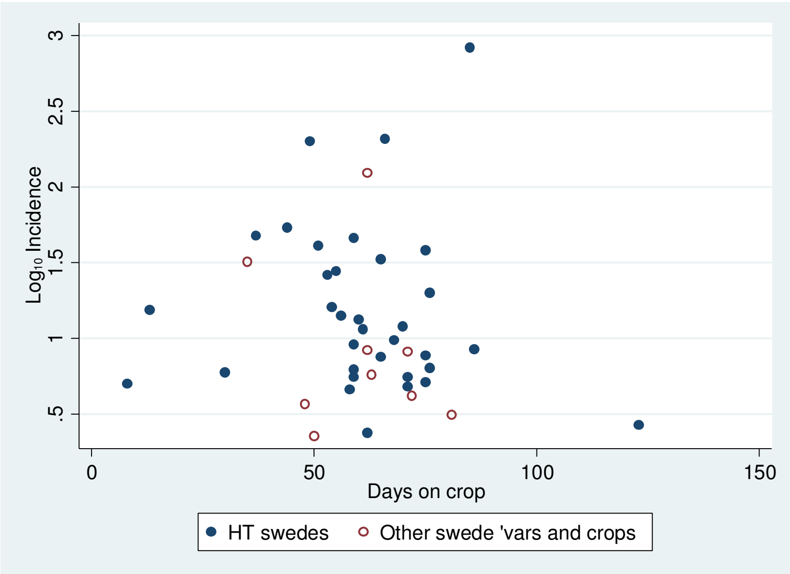


Figure 15 Wintering on the milking platform (WMP) $\log_{10}$ cumulative incidence (per 1000 animals) versus days on crop for HT® swedes (blue closed circles) and other swede varieties and crops (red open circles) summarised from data collected during a survey of dairy farmer wintering practices in Southland and south Otago during winter/spring 2014.

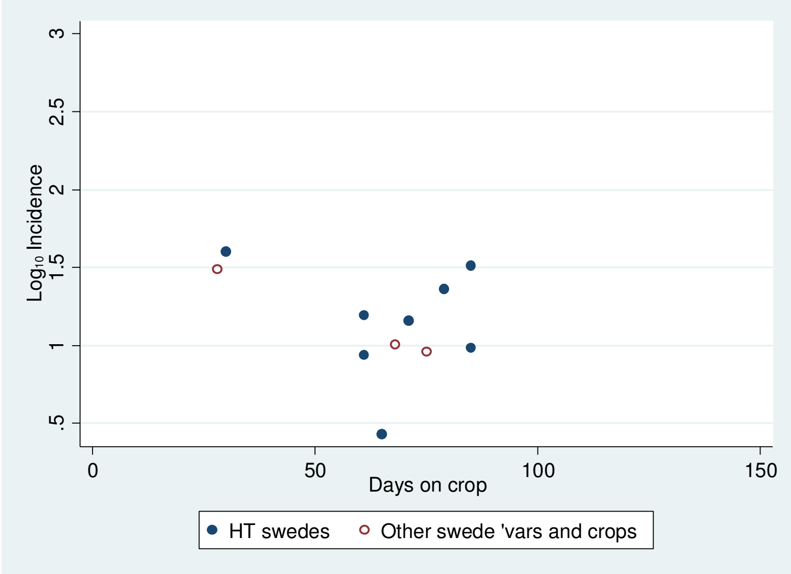
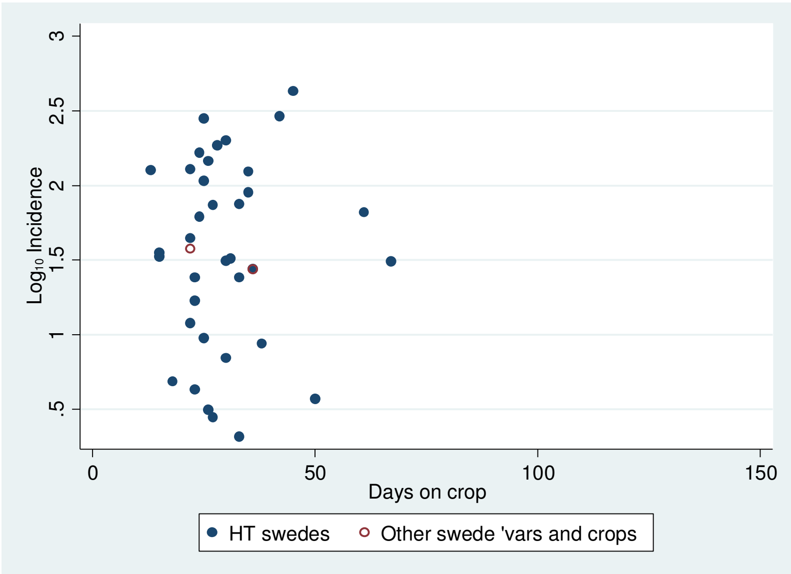


Figure 16 Crop fed during calving and early lactation (CMP) $\log_{10}$ cumulative incidence (per 1000 animals) versus days on crop: HT® swedes (blue closed circles) and other swede varieties and crops (red open circles) summarised from data collected during a survey of dairy farmer wintering practices in Southland and south Otago during winter/spring 2014.



In simple sub-acute toxicoses, for example exposure to a toxic chemical where there is continuous steady exposure, one would expect that cumulative incidence (i.e. risk) would be correlated with the period of exposure. That this did not occur suggests that outcomes, in terms of morbidity and mortality, were modulated by other factors; i.e. evidence of multi-factorial causation. One key determinant shown in the survey of swedes is the glucosinolate concentration of plant material: there was variation between different parts of the plant and higher concentrations in the reproductive elements (see Section 7).

The cumulative probability distribution of the incidence rate[i] (per 1000 animals per month) is presented in Figure 17. As seen for the cumulative incidences, these are right skewed, especially for the WOF scenario. Again the averages will be highly influenced by some very high values and thus the medians are listed in Table 8. Of note is the median CMP value was 7 to 8 times that seen in WOF and WMP.

Figure 17 Incidence rate (IR) per 1000 animals per month for the three wintering scenarios (wintering off farm, WOF; wintering on milking platform, WMP; and swede crops fed during calving and early lactation, CMP) identified during the swede associated liver disease event in Southland in winter/spring 2014. Graphs show the cumulative probability[j] versus the incidence rate values.

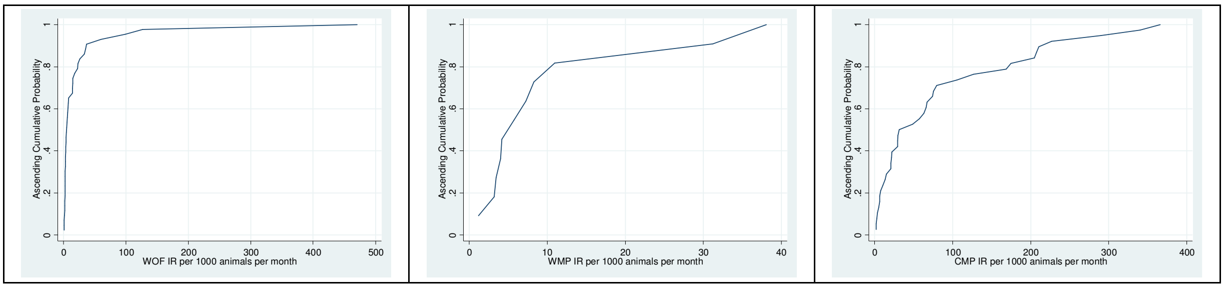


Table 8 Incidence rate per 1000 animals per month for the three wintering scenarios (wintering off farm, WOF; wintering on milking platform, WMP; and swede crops fed during calving and early lactation, CMP) identified during the swede associated liver disease event in Southland in winter/spring 2014. Table shows number of different groups (n), median and 95% confidence limits (CL) of the median values.

Scenario	n	Median	Low 95 CL	Upper 95 CL
WOF (Wintering off farm)	43	5	3	11
WMP (Wintering on milking platform)	11	6	3	17
CMP (Crop fed calving and early lactation)	38	40	21	75

In Figure 18, box plots of the log transformed data are presented. Of note is that:

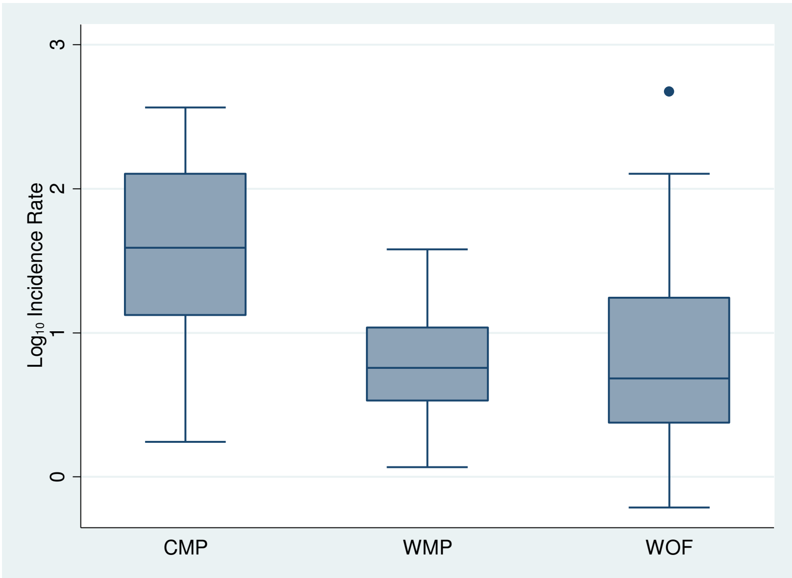
- log transformation has yielded symmetrical distributions (there is one outlier in the WOF series). In all three scenarios the data passed checks for normality (P = 0.178, 0.598 and 0.118 for CMP, WMP and WOF[k], respectively).
- CMP incidence rates are generally higher than those seen in WOF and WMP.

[i] ((Number ill + Number dead)/(Days at risk * (Number at start – ((Number at start – (Number ill + Number dead))/2)*1000*28
[j] See glossary
[k] Outlier deleted

Using log transformed data, with the one outlier in the WOF scenario deleted, an ANOVA was conducted, followed by a pairwise comparison using the t-test. The ANOVA returned a highly significant P-value ($P < 0.001$); CMP versus WOF also returned a significant difference (P = < 0.001) as did CMP versus WMP ($P = 0.002$). WOF versus WMP returned a P-value of 1.000.

Considering the WOF scenario, the effect of the factor 'HT® swedes' on IR is not significant ($P = 0.200$).

Figure 18 Box plots of $\log_{10}$ incidence rate (per 1000 animals per month) during calving/early lactation (CMP) and for wintering on the milking platform (WMP) and wintering off farm (WOF) in Southland and south Otago during winter/spring 2014.



6 An investigation of risk factors associated with the cases

6.1 Background

It is recognised that health outcomes (including negative ones) in both individuals and populations are the result of a number of factors[I] that come together, often in a unique way, in time and space. Various terms have been coined to describe this, such as 'causal webs' and 'multi-factorial causation'. For this reason the expert group who had been brought together recommended that a wide ranging investigation should be launched to explore possible causal factors.

In epidemiology, the term 'risk factor' is used to describe 'any attribute, characteristic or exposure of an individual (or herd) that increases the likelihood of developing a disease or injury'. A common method used to identify risk factors is to conduct a 'retrospective observational study'. The crux of such an investigation is as follows:

- Draw up a list of items (i.e. factors) that could be involved in the disease outbreak
- Identify a representative group of affected herds and herds that have not been affected
- By way of field investigations, interviews, analyses of records etc. determine whether or not each of the herds were 'exposed' to each of the factors
- Conduct a statistical analysis to determine if any of the differences between affected and non-affected groups could be explained by chance; i.e. significance testing
- If a significant effect is found, determine the strength of the association.

The investigation was restricted to farmers that had fed swede crops during the winter and/or spring of 2014.

Multiple interviewers were required to complete the survey; to ensure consistency all interviewers undertook training before farmer contact. Interviews were conducted between early November and mid-December 2014. Participants' veterinarians were also contacted and a second in-depth survey was completed with the participants' graziers and crop establishment and spray contractors between January and March 2015.

6.2 Key outcomes

6.2.1 Risk factors

The potential risk factors that were established are listed in the following tables. The broad divisions are items related to the swede crops (Table 9), to management of cattle before, during and after feeding of the crops (Table 10) and to the use of herbicides on the crops (Table 11).

[I] Commonly called the "determinants" of a disease

Table 9 Swede crop factors documented from a survey of farmers feeding swedes in Southland and south Otago during winter/spring 2014.

Type of crop exposure
Swede variety exposure
Swede area
Prior crop
Paddock topography
Paddock aspect
Ground preparation – lime
Growing phase – urea
Ground preparation - agrichemical use
Growing phase – agrichemical use
Fertiliser – trace elements
Crop disease
Crop yields

Table 10 Animal management factors documented from a survey of farmers feeding swedes in Southland and south Otago during winter/spring 2014.

Cow movement off farm – stand-off
Cow movement off farm – transport
Cow movement off farm – walking time
Wintering mob size
Diet transition – on arrival
Special diet transition process
Swede dry matter week 1
Diet transition - crop/baleage ratio
Diet transition – change in crop/baleage
Frequency shifting break wire
Duration of feeding
Crop maturity at feeding - immature
Crop maturity at feeding – mature
Crop maturity at feeding - flowering
Crop maturity at feeding – flowering
Swede ration offered(kg/day) – HT® variety
Swede ration offered(kg/day) – not HT® variety
Swede ration offered(kg/day) – all varieties
Swede ration offered(% of DM) – HT® variety
Swede ration offered(% of DM) – not HT® variety
Method of feeding supplements
Cow feeding preference – swede
Animal breakouts
% dry matter as swede in diet, HT® as factor

Table 11 Herbicide factors documented from a survey of farmers feeding swedes in Southland and south Otago during winter/spring 2014.

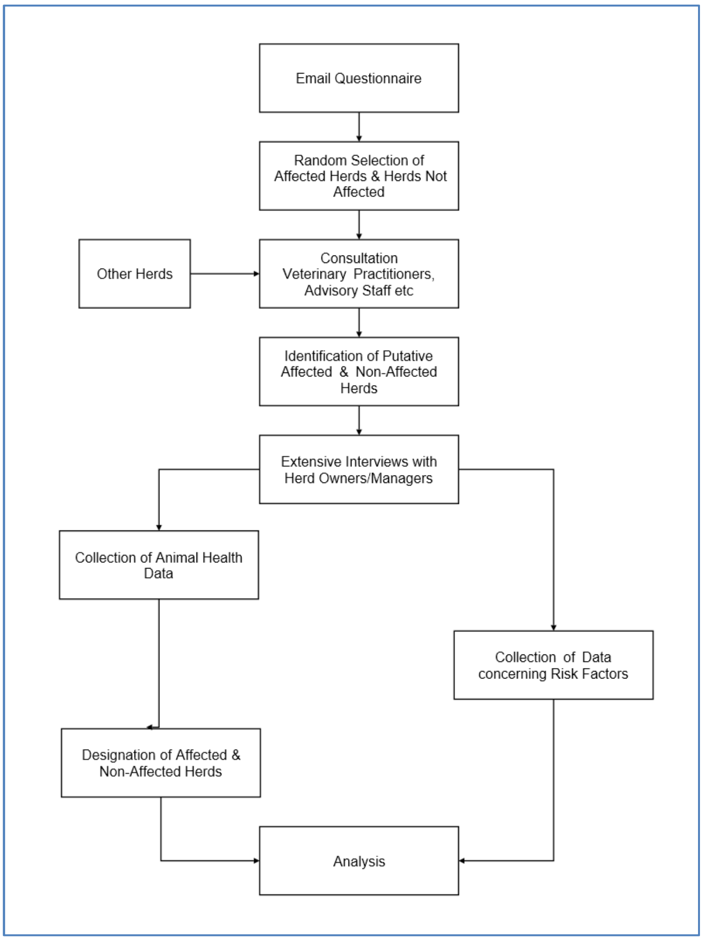
Glyphosate use during ground preparation and planting
Chlorsulfuron use at planting
Chlorsulfuron use during growth phase (HT® crop only)
Chlorsulfuron use during growth phase (HT® crops only)
Chlopyrifos use during growth phase

In addition an analysis was undertaken of climatic factors occurring over the period when illness and deaths were reported, as compared with long-term averages.

6.2.2 Herds affected and not affected

The pathway that was used to identify a representative group of both affected herds and herds where illness and deaths had not occurred while grazing swede groups is illustrated in Figure 19. In total there were 87 out of 138 (63%) in the former group and 51 (37%) in the latter.

Figure 19 The pathway in the investigation of disease risk factors followed to select representative samples of both 'affected herds[m]' and 'non-affected herds' for surveying in relation to the swede associated liver disease event in winter/spring 2014 .



[m] Where illness and deaths in cattle grazing swedes had occurred

6.2.3 Results of the initial screen for risk factors

Each one of the scenarios (WOF, WMP and CMP) was investigated separately. In a follow-up phase, analyses of combined data were also undertaken.

An in-depth analysis of the data collected was undertaken. In some areas, this was hampered by missing values. However, despite this wide ranging investigation, only a limited number of likely risk factors were identified, as follows.

In the CMP scenario, positive associations between disease and the feeding of HT® swedes (as compared with feeding other crops) was detected and significance testing suggested that this was highly unlikely to be due to chance (i.e. $P < 0.05$). This was not evident in the WOF and WMP scenarios

Marginal associations ($0.05 < P < 0.15$) were also found between disease and the proportion of swede crop in rations in both the CMP and WOF scenarios.

Inspection of the climate data support the view that climatic conditions in winter and spring 2014 differed from published values for ten year average data. Air temperatures from April through June, as well as in August, were generally warmer than normal, at times and in places by as much as 1.5℃. The pattern of monthly rainfall totals also differed from the ten year average with greater than normal amounts in April, May, and July. Weather stations at Lumsden and Gore both reported that total rainfall for the six months from April to September 2014 (inclusive) was 33% greater than the ten-year average (Appendix 10.3).

6.2.4 Results of a follow-up analysis of putative risk factors

Following on from the initial screen, a more detailed analysis of the putative risk factors was undertaken, in particular using exact statistical techniques. Some of the contingency tables used in the screen were unbalanced and cell contents were sparse. In these circumstances exact methods provide more reliable outcomes.

In this analysis, only those herds in which the numbers of animals ill or dead, number exposed etc. were available were included (i.e. six herds were excluded).

A traditional case-control approach was used in this analysis, where cases were defined as herds where illness or death had occurred and controls where none had been observed. This was set separately for each scenario (CMP, WOF and WMP).

Three types of ‘exposures’ were investigated;

- First, HT® swedes versus all other crops
- Second, HT® swedes versus other swede crops
- Third, all All swedes versus other crops.

These are referred to as ‘exposure 1’, ‘exposure 2’ and ‘exposure 3’ respectively.

A summary of the results is presented in Table 12. This information is also illustrated in the following dot graph (Figure 20). In addition, the outputs from the statistical package are presented at the end of this section (6.2.4.1).

Arguably the most important result is the confirmation of a statistically significant ($P < 0.001$) association between disease and HT® swedes (exposure code 1 in the table and figure) in the CMP scenario (calving/early lactation). The odds ratio, a measure of the strength of this association, is moderately high (24.0), but the 95% confidence limits are very wide (4.7 to 225.9). The very wide confidence limits for the odds ratio are a result of the very unbalanced case data and the moderate number cases overall (see Figure 21).

In the CMP scenario, the association between disease and HT® versus other swede varieties (exposure code 2) also returned a highly significant result (P < 0.001). All of the CMP 'case' swede types were HT® and, therefore, it is not possible to calculate an odds ratio. With regard to the association between disease and all swede types, the P-value was not significant (0.418).

In the WOF scenario, a statistically significant association (P = 0.012) was evident between disease and HT® crops. However, this was not the case (P = 0.173) when this was limited to a comparison between HT® and other swede crops (exposure code 2). When an exposure to 'any swede' crop was tested (exposure code 3), statistical significance was achieved (P = 0.043). This is reflected in the 95% confidence interval of the odds ratio (i.e. the lower limit is close to 1.0). This result suggests that swedes, in general, compared with other crops, posed a risk pre-calving in 2014. However, caution is required as herds were selected for this study with reference to being fed swedes (i.e. not all crops), and this may have affected the outcome.

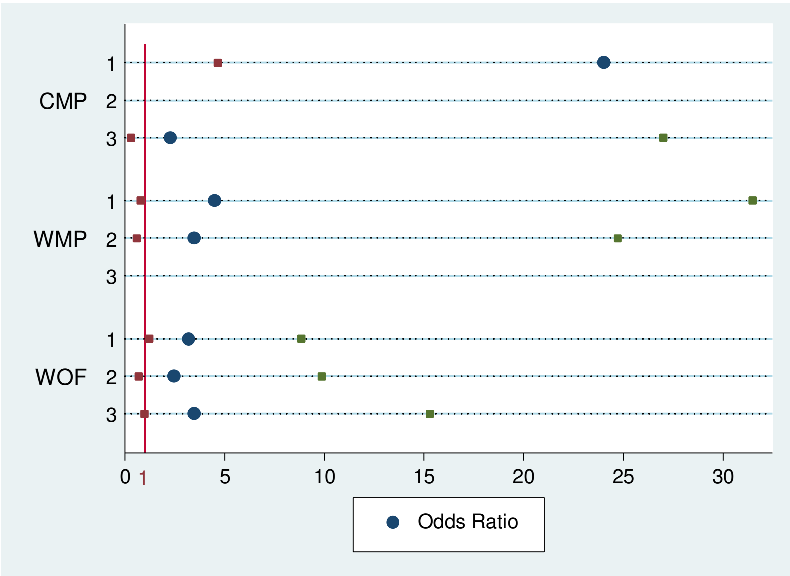
In the WMP scenario, a trend (i.e. a marginally (P = 0.074) significant association) between disease and HT® crops was also evident. But, as with WOF, when this was limited to a comparison between HT® and other swede types, a P-value of 0.152 was returned. As with CMP, the test of the association between disease and all swede type was not significant (P = 0.303). All the case crops were swedes and, therefore, it was not possible to calculate an odds ratio.

Table 12 Results of the investigation into the association between disease (i.e. case/control herds) and exposure to swede and other crops, under three scenarios (crops fed during calving/early lactation (CMP), crops fed wintering on the milk platform (WMP), and crops fed wintering off farm (WOF)) during winter/spring 2014 in Southland and south Otago.

Scenario	Exposure Type		Exact P-value	Odd Ratio (OR)	Lower OR CL	Upper OR CL
CMP	(1)	HT® versus all other crops	< 0.001	24.0	4.7	225.9
CMP	(2)	HT® versus other swede variants	< 0.001	not available		
CMP	(3)	All swedes versus other crops	0.418	2.3	0.3	27.0
WMP	(1)	HT® versus all other crops	0.074	4.5	0.8	31.5
WMP	(2)	HT® versus other swede variants	0.152	3.5	0.6	24.7
WMP	(3)	All swedes versus other crops	0.303	not available		
WOF	(1)	HT® versus all other crops	0.012	3.2	1.2	8.9
WOF	(2)	HT® versus other swede variants	0.173	2.5	0.7	9.9

Scenario	Exposure Type	Exact P-value	Odd Ratio (OR)	Lower OR CL	Upper OR CL
WOF	(3) All swedes versus other crops	0.043	3.5	1.0	15.3

Figure 20 Results of the investigation into the association between disease presence/absence (case/control herds) and exposure to swede and other crops; under three scenarios (crops fed during calving/early lactation (CMP), crops fed wintering on the milk platform, and crops fed wintering off farm (WOF)) during winter/spring 2014 in Southland and south Otago; see Section 6.2.4[n] above for description of exposure codes; the lower (red square) and upper (green square) 95% confidence limits of the odd ratios (blue circle) are shown.



Two useful parameters can be estimated from an unmatched case control study[8].

The 'Attributable Fraction exposed' (AF_e) is the proportion of disease in exposed individuals (herds) that is due to the exposure. The 'Attributable Fraction population' (AF_p) indicates the proportion of disease in the whole population (all herds) that is attributable to the exposure.

These parameters have been estimated for the statistically significant ($P \leq 0.05$) associations between disease and exposure (Table 13). This suggests that feeding HT® swedes during calving and early lactation is responsible for much of the observed disease. Assuming the case and control herds in this analysis are representative samples of all case and control herds (i.e. no selection bias), the data indicate that HT® swedes were responsible for around 90% of disease over the period of late pregnancy, calving and early lactation. However, if selection bias is, unintentionally present, with more focus on HT® swedes, then the percentage may be overestimated.

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[n] Three types of 'exposures' were investigated; first HT® swedes versus all other crops, second HT® swedes versus other swede crops, and third all swedes versus other crops. These are referred to as 'exposure 1', 'exposure 2' and 'exposure 3' respectively.

With respect to the pre-calving period (WOF), the estimates have very wide 95% confidence intervals, and are therefore unreliable. More focussed work in this area would be of value.

Table 13 Estimates of the 'Attributable Fraction exposed' (AF_e) and 'Attributable Fraction population' where a significant (P ≤ 0.05) association between disease and an exposure was found in data collected during a survey of farmers in Southland and south Otago following the swede associated liver disease event in winter/spring 2014.

Scenario	Exposure Type		AF_e Estimate	AF_e Lower 95% CL	AF_e Upper 95% CL	AF_p
CMP	(1)	HT® versus all other crops	96%	79%	99%	91%
WOF	(1)	HT® versus all other crops	68%	18%	89%	55%
WOF	(3)	All swedes versus other crops	71%	0%	93%	65%

6.2.5 Influence of prior disease status and crop exposure

As outlined in section 4.2 most of the *Brassica* spp. toxicoses fall into a moderate to long term category, in contrast to such acute syndromes as botulism. There is, therefore, a valid question concerning whether or not pre-calving disease or feeding swedes (HT® and not HT®) exposure are risk factors for disease in the CMP scenario.

To investigate this, a subset of the data was generated consisting of herds where there was both CMP and WOF or WMP survey returns. Flags were set for disease occurrence, exposure to swedes and exposure to HT® swedes in either of the WOF or WMP scenarios.

The subset contained 63 herds. No statistical association was found between disease in the CMP scenario and disease during wintering (P = 0.203), exposure to swedes during wintering (P = 0.763) and exposure to HT® crops during wintering (P = 1.000).

6.2.6 Statistical outputs from Stata[o]

Figure 21 Scenario = CMP, Exposure = HT® swedes versus all other crops.

	Exposed	Unexposed	Total	Proportion Exposed
Cases	36	2	38	0.9474
Controls	15	20	35	0.4286
Total	51	22	73	0.6986

	Point estimate	[95% Conf.	Interval]	
Odds ratio	24	4.658742	225.8512	(exact)
Attr. frac. ex.	.9583333	.7853498	.9955723	(exact)
Attr. frac. pop	.9078947			

1-sided Fisher's exact P = 0.0000
2-sided Fisher's exact P = 0.0000

Figure 22 Scenario = CMP, Exposure = HT® swedes versus other swede varieties.

	cmp_sht Exposed	Unexposed	Total	Proportion Exposed
Cases	36	0	36	1.0000
Controls	15	16	31	0.4839
Total	51	16	67	0.7612

	Point estimate	[95% Conf.	Interval]
Odds ratio	.	9.366704	.
Attr. frac. ex.	.	.8932389	.
Attr. frac. pop	.		

1-sided Fisher's exact P = 0.0000
2-sided Fisher's exact P = 0.0000

Note: Exact confidence levels not possible with zero count cells.

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[o] Stata version 14.0, StataCorp, 4905 Lakeway Drive, College Station Texas, USA

Figure 23 Scenario = CMP, Exposure = All swedes versus other crops.

	Exposed	Unexposed	Total	Proportion Exposed
Cases	36	2	38	0.9474
Controls	31	4	35	0.8857
Total	67	6	73	0.9178

	Point estimate	[95% Conf. Interval]		
Odds ratio	2.322581	.3052239	27.01092	(exact)
Attr. frac. ex.	.5694444	-2.276284	.9629779	(exact)
Attr. frac. pop	.5394737			

1-sided Fisher's exact P = 0.2982
2-sided Fisher's exact P = 0.4177

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Figure 24 Scenario = WOF, Exposure = HT® swedes versus all other crops.

	Exposed	Unexposed	Total	Proportion Exposed
Cases	34	9	43	0.7907
Controls	33	28	61	0.5410
Total	67	37	104	0.6442

	Point estimate	[95% Conf. Interval]		
Odds ratio	3.205387	1.224706	8.850597	(exact)
Attr. frac. ex.	.6880252	.1834772	.8870133	(exact)
Attr. frac. pop	.5440199			

1-sided Fisher's exact P = 0.0073
2-sided Fisher's exact P = 0.0122

Figure 25 Scenario = WOF, Exposure = HT® swedes versus other swede varieties.

	Exposed	Unexposed	Total	Proportion Exposed
Cases	34	5	39	0.8718
Controls	33	12	45	0.7333
Total	67	17	84	0.7976

	Point estimate	[95% Conf. Interval]		
Odds ratio	2.472727	.7049601	9.876567	(exact)
Attr. frac. ex.	.5955882	-.4185199	.8987502	(exact)
Attr. frac. pop	.5192308			

1-sided Fisher's exact P = 0.0954
2-sided Fisher's exact P = 0.1732

Figure 26 Scenario = WOF, Exposure = All swedes versus other crops.

	Exposed	Unexposed	Total	Proportion Exposed	
Cases	39	4	43	0.9070	
Controls	45	16	61	0.7377	
Total	84	20	104	0.8077	
	Point estimate		[95% Conf.	Interval]	
Odds ratio	3.466667		.9931138	15.28546	(exact)
Attr. frac. ex.	.7115385		-.0069339	.9345784	(exact)
Attr. frac. pop	.6453488				

1-sided Fisher's exact P = 0.0258
2-sided Fisher's exact P = 0.0425

Figure 27 Scenario = WMP, Exposure = HT® swedes versus all other crops.

	Exposed	Unexposed	Total	Proportion Exposed	
Cases	8	3	11	0.7273	
Controls	10	17	27	0.3704	
Total	18	20	38	0.4737	
	Point estimate		[95% Conf.	Interval]	
Odds ratio	4.533333		.8034785	31.46401	(exact)
Attr. frac. ex.	.7794118		-.2445884	.9682177	(exact)
Attr. frac. pop	.5668449				

1-sided Fisher's exact P = 0.0499
2-sided Fisher's exact P = 0.0741

Figure 28 Scenario = WMP, Exposure = HT® swedes versus other swede varieties.

	Exposed	Unexposed	Total	Proportion Exposed	
Cases	8	3	11	0.7273	
Controls	10	13	23	0.4348	
Total	18	16	34	0.5294	
	Point estimate		[95% Conf.	Interval]	
Odds ratio	3.466667		.5976254	24.71618	(exact)
Attr. frac. ex.	.7115385		-.6732891	.9595407	(exact)
Attr. frac. pop	.5174825				

1-sided Fisher's exact P = 0.1086
2-sided Fisher's exact P = 0.1519

Figure 29 Scenario = WMP, Exposure = All swedes versus other crops.

	wmp_swe Exposed	Unexposed	Total	Proportion Exposed
Cases	11	0	11	1.0000
Controls	23	4	27	0.8519
Total	34	4	38	0.8947

	Point estimate	[95% Conf.	Interval]
Odds ratio	.	.4420236	.
Attr. frac. ex.	.	-1.262323	.
Attr. frac. pop	.		

1-sided Fisher's exact P = 0.2378
2-sided Fisher's exact P = 0.3026

Note: Exact confidence levels not possible with zero count cells.

7 Glucosinolates in Swedes

7.1 Background

Initial feedback from the field about the nature of the disease observed in cattle grazing swedes pointed strongly in the direction of a toxicosis arising from derivatives of the GSLs and, in particular, both nitriles and epithionitriles.[1]. SMCO concentration was included in the analysis suite, as a precaution, to determine if the concentrations were sufficiently high to cause toxic effects and, therefore, anaemia.

It was also recognised that the collection of plant material was less than ideal: samples could only be collected in September, when the event was well advanced (i.e. between the 09-11 and the 16-19 September 2014), and, in many situations, particularly where no animal health problems had been observed, the swedes had all been grazed. In addition, with the difficulties of using liquid nitrogen (- 80°C) in field conditions, DairyNZ staff focussed on collecting the reproductive plant parts that were thought most likely to be causing the issue and few bulb samples were collected.

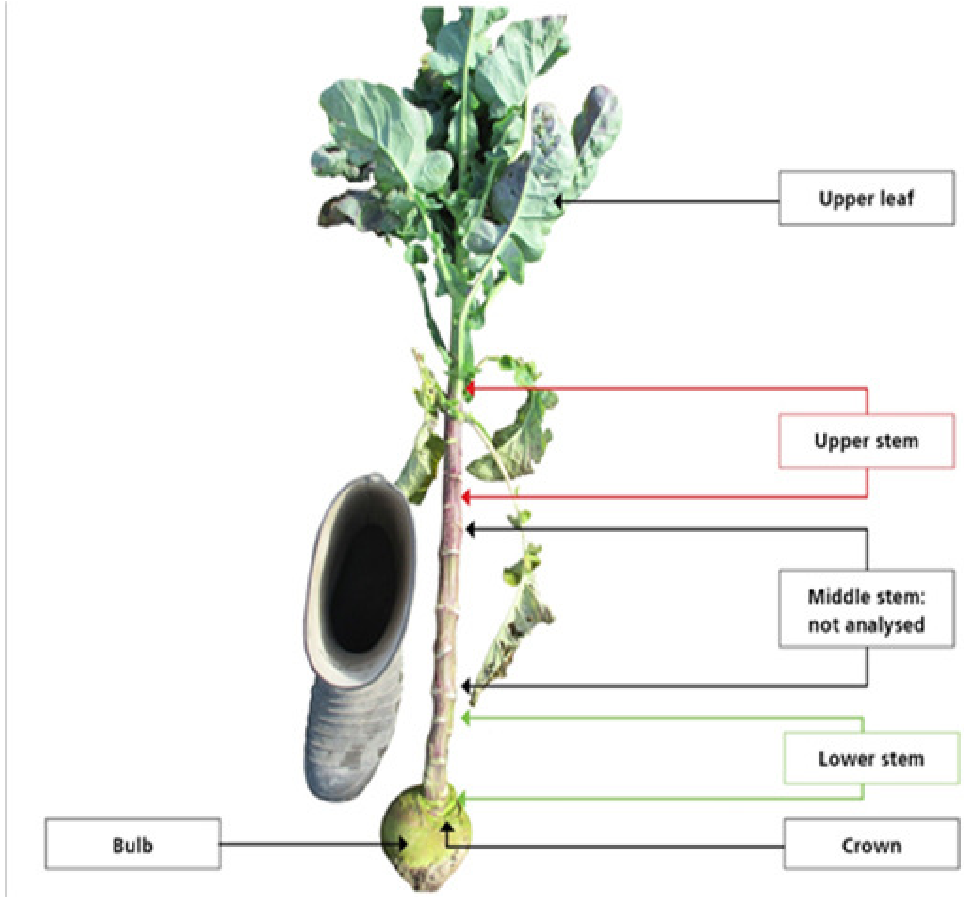
A decision was made to limit plant analysis to the GSL concentrations and SMCO. It was confirmed by Dr Mark Collett, Massey University, that the suite included the entire candidate GSLs of interest for his toxicology research.

The objectives of the plant analysis were:

- To determine if the concentration of GSLs in the plant parts collected (i.e. bulb crown, lower leaf, lower stem, upper leaf, upper stem and flower) in HT® swedes compared with other varieties, particularly where adverse animal health effects were reported
- To establish a validated method for testing individual GSL and SMCO concentrations, by an accredited commercial laboratory in New Zealand for plant breeders and/or farmers to assess the potential toxicity of a swede crop.

Figure 30 shows the plant parts that were dissected and analysed for GSL concentrations. Both sub-samples for each plant component were analysed for the selected farms.

Figure 30 shows the plant parts that were dissected and analysed for glucosinolate concentrations. The photo is that of a typical swede from the 2014 winter, which has entered the reproductive growth phase (i.e. the plant has developed an elongated stem, new leaves and seed heads or flowers may have formed. The term 'bolted' refers to such a status).



To meet these objectives individual GSL concentrations, rather than a single analysis for total GSLs, were required. With progoitrin expected to be the largest single GSL, a single analysis for total GSLs could mask the change in individual GSL profiles and therefore a potential cause of the toxicity after ingestion. Also, selection priority for analysis was given to swede samples from farms with accompanying animal samples.

In December 2014, Hill Laboratories was contracted to develop a method for testing for the 29 total analytes (28 GSLs and SMCO; Appendix 2) for a range of GSL concentrations that were expected in be present in the swede parts collected. It was expected that differences in GSL concentrations would be the greatest between the swede bulb and seed heads or flowers. The laboratory was contracted to explore several extraction procedures during the method development process to ensure a robust outcome.

The analysis of samples was subdivided into three subsets; an initial subset of farm samples where the duplicate samples were analysed to ascertain if single samples could be analysed for the balance of the farms. This was followed by a second submission of samples where analysis on duplicate samples was also completed. It was concluded from the preliminary analysis of these two sets of data that the plant analysis supported the hypothesis and that further analysis of additional samples was unlikely to change the advisory messages. It was assumed that the samples from the:

- Three farms where severe ill-health had been observed when cattle grazed HT® swedes; and
- Four farms where no ill-health had been observed when cattle grazed non-HT® swedes

were most likely to provide results with the widest range in GSL concentrations. Additional samples were included in the analysis with preference generally given to farms where both plant and animal samples were available and the in-depth survey had been completed.

A summary of the sub-set of farms where the swede samples were analysed for individual GSL and SMCO concentrations is presented in Table 14. These samples were collected as part of the initial response activity (section 3.1.2). It should not be inferred from Table 14 that there were no health issues with non-HT® swedes.

Table 14 A summary of the nineteen farms that assisted by providing a range of samples (crop and/or animal) in the initial phase of the investigation into the swede associated liver disease event in Southland and south Otago during winter/spring 2014. The swede material collected from the farms highlighted in green, were analysed for individual glucosinolate and SMCO concentrations.

Farm	Swede variety sampled	Ill health observed	Date collected Sept. 2014	Farm Location
1	HT	Yes - severe	09-11	Eastern Southland
2	HT	Yes - severe	09-11	Northern Southland
3	HT	Yes	09-11	Central Southland
4	HT	Yes	09-11	Central Southland
5	HT	No	09-11	Western Southland
6	HT	No	16-19	Eastern Southland
7	HT	No	16-19	Central Southland
8	Domain	No	16-19	Eastern Southland
9	HT	Yes	16-19	Central Southland
10	Aparima Gold	No	16-19	West Otago
11	Aparima Gold	No	16-19	Eastern Southland
12	HT	Yes - severe	16-19	Central Southland
13	HT	Yes	16-19	Central Southland
14	HT	No	16-19	Northern Southland
15	Aparima Gold	No	16-19	Eastern Southland
16	HT	Yes	16-19	Western Southland
17	HT	Yes	09-11	Eastern Southland
18	HT	Yes	16-19	Western Southland
19	HT	Yes	16-19	Southland

7.2 Key findings

7.2.1 Total glucosinolate concentration

The overall mean total GSL concentration across all farms was 37.59 µmol/g dry weight, with 95% confidence limits of 29.39 and 45.80. However, results from some plant parts were not available and, thus, this estimate is somewhat unreliable.

As noted in the review of the scientific literature (see Section 4.2.3), two major factors affecting the concentration of GSLs are the plant part and cultivar (i.e. genotype), and this was a major focus of this survey. The breakdown of concentrations by plant part and by swede variety (i.e. HT® and non-HT®) are listed in Table 14 and illustrated in Figures 31 and 32. To summarise the outcomes:

- There were significant differences ($P<0.001$) between:
    - different plant parts
    - HT® and non-HT® swede varieties.
- For all plant parts except bulb/crown, the GSL concentration was higher in HT® than non-HT® swedes
- In non-HT®, swedes bulb/crown and lower leaf were similar, then there was a steady increase through lower stem, upper leaf, upper stem and flower
- In HT® swedes, there was an increase from bulb/crown, but upper stem, upper leaf and flower were similar

Table 14 Mean concentration of total glucosinolates (µmol/g dry matter) and minimum and maximum by plant part and by swede variety. A and B field replicate samples.

Variety	Plant Part	Mean	Minimum	Maximum
All	Bulb/Crown	20.21	15.05	25.87
	Lower Leaf	30.37	9.60	50.00
	Lower Stem	33.63	8.81	51.01
	Upper Stem	47.86	6.99	91.71
	Upper Leaf	42.43	16.90	67.83
	Flower	44.96	10.90	81.44
HT®	Bulb/Crown	21.27	16.93	25.87
	Lower Leaf	35.73	19.91	50.00
	Lower Stem	39.53	24.22	51.01
	Upper Stem	57.78	37.53	91.71
	Upper Leaf	49.68	40.92	67.83
	Flower	50.18	18.35	81.44
Other (not HT®)	Bulb/Crown	18.61	15.05	23.40
	Lower Leaf	18.99	9.60	37.02
	Lower Stem	23.30	8.81	33.97
	Upper Stem	29.26	6.99	44.25
	Upper Leaf	26.12	16.90	32.54
	Flower	31.03	10.90	45.15

Figure 31 Mean concentration of total glucosinolates (µmol/g dry matter) and minimum and maximum by swede variety (HT® (HT) or non HT® (NHT) and by plant part (bulb/crown, lower leaf, lower stem, upper stem, upper leaf, flower).

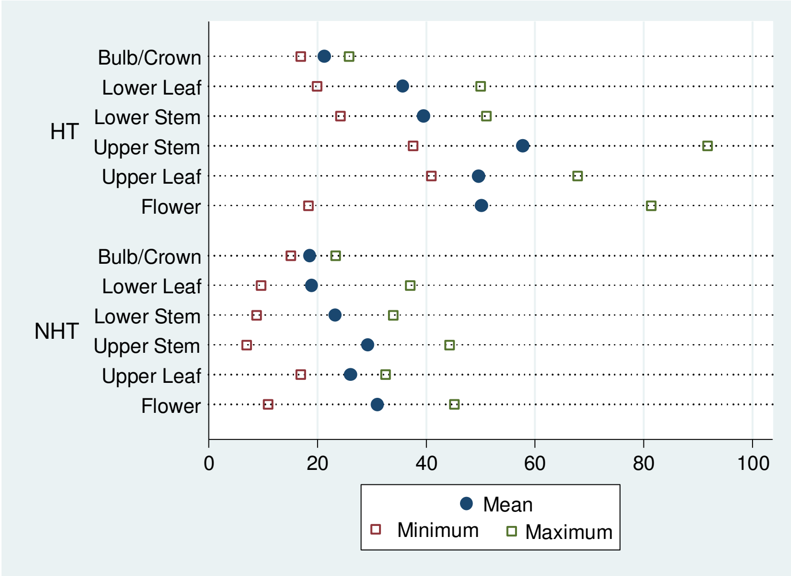
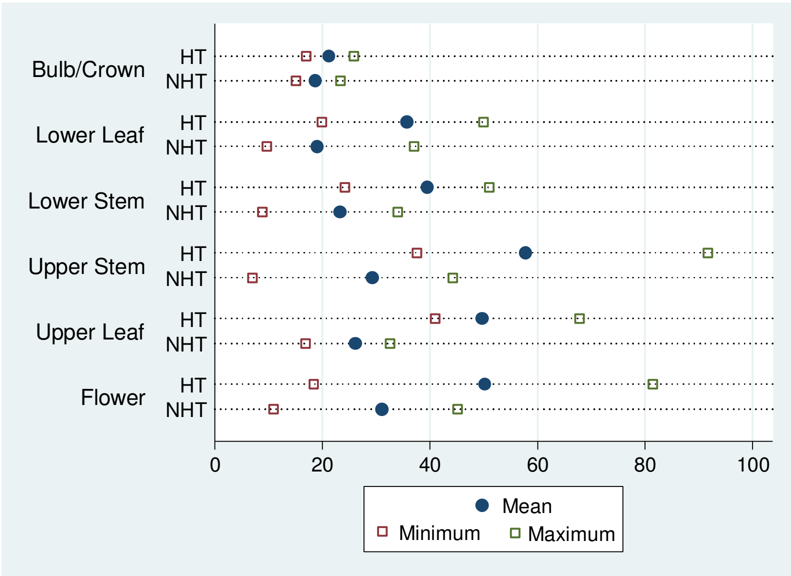


Figure 32 Mean concentration of total glucosinolates (µmol/g dry matter) and minimum and maximum by plant part (bulb/crown, lower leaf, lower stem, upper stem, upper leaf, flower) and by swede variety (HT® (HT) or non HT® (NHT).



The analysis of the association between concentration and plant part/cultivar yielded a coefficient of determination[p] (R^2) of only 55.29%. Further, during the preliminary exploratory data analysis it was observed that the 'cultivar-plant part' relationship varied considerably on different farms. This is illustrated in Figures 33 and 34 which show the concentration in plant parts on HT® and non-HT® farms.

[p] The proportion of the variance in the dependent variable that is predictable from the independent variables.

Figure 33 Concentration of total glucosinolates (µmol/g dry matter) by plant part (bulb/crown, lower leaf, lower stem, upper stem, upper leaf, flower) and farm where HT® swede crops were grown.

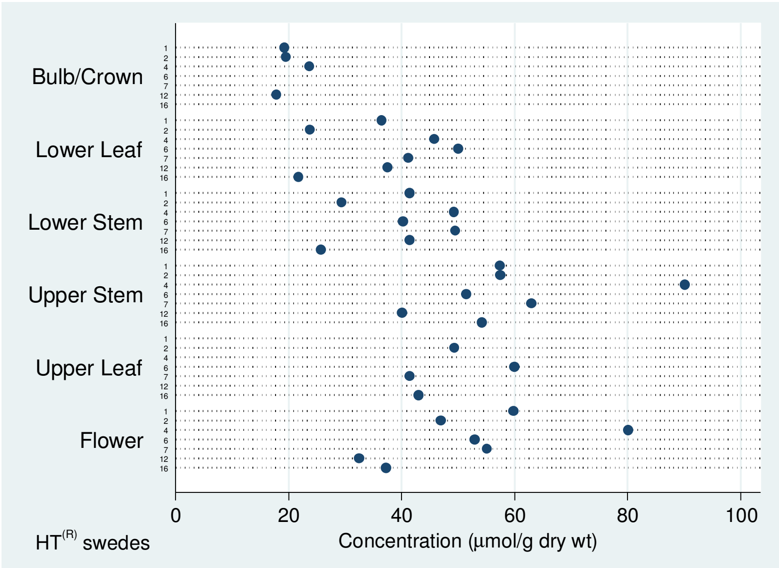
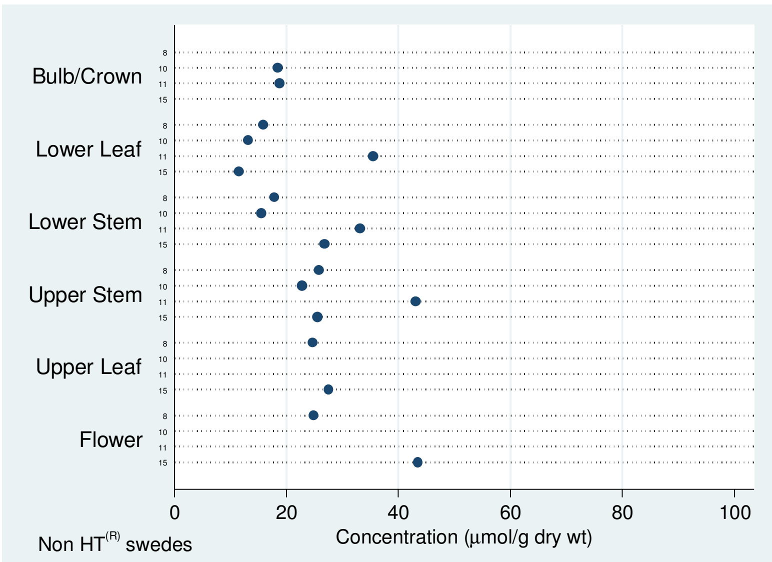


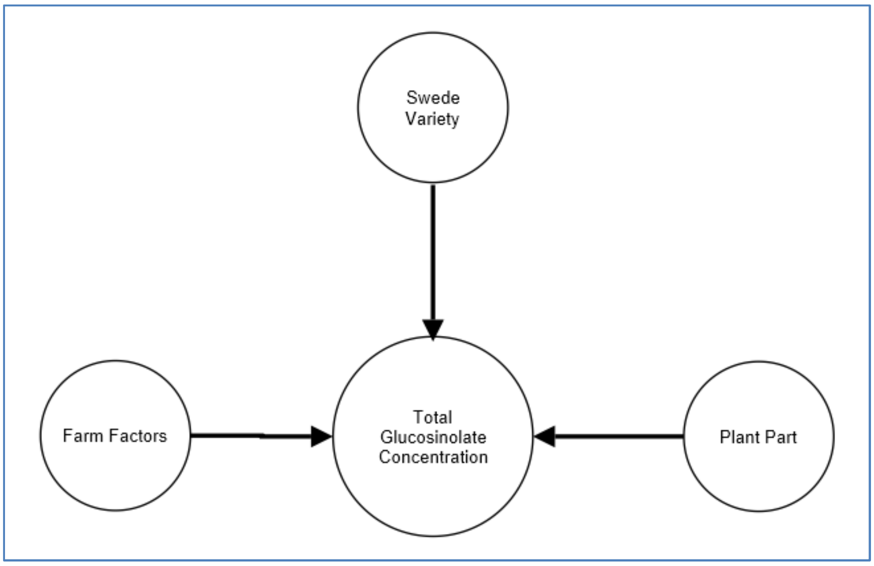
Figure 34 Concentration of total glucosinolates (µmol/g dry matter) by plant part (bulb/crown, lower leaf, lower stem, upper stem, upper leaf, flower) and farm where swede crops other than HT® were grown.



That only a moderate amount of variation was explained by plant part/cultivar and further investigations/analyses such as variance components analysis (data not shown), suggests that there is substantial variation between farms growing the same cultivar. A randomised method was used to select a representative sample of plant parts from swede crops and thus one would expect that the variation within farms would not be large. This is an area that will be subject to further investigation.

A diagrammatic representation of the overall outcome is presented in Figure 35. Plant part, cultivar (i.e. HT®) and 'farm' (i.e. differences arising from crop management, soils, climate etc) have major effects.

Figure 35 Diagrammatic representation of the factors affecting the concentration of total glucosinolate concentration in swedes.

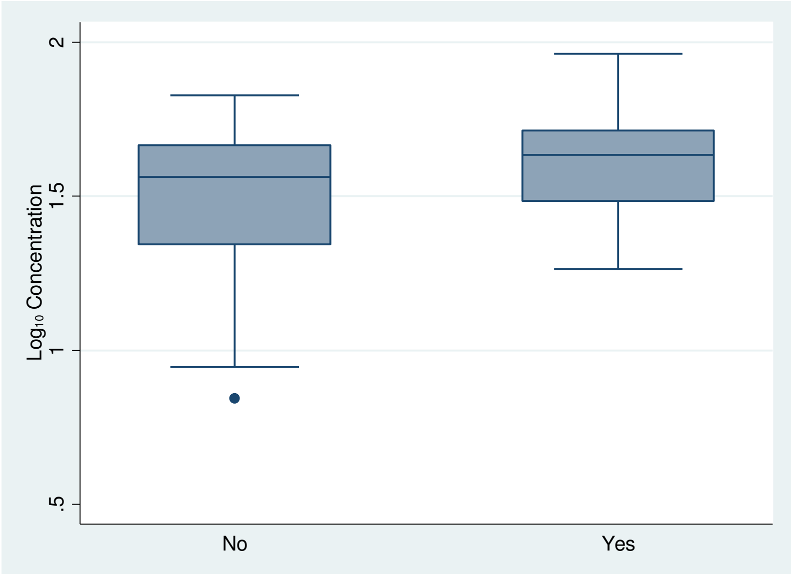


7.2.2 Total glucosinolates on affected and non-affected farms

On five of the eleven properties where swedes were collected for analysis, illness and/or deaths had been observed. Unfortunately, the analysis to determine if there were significant differences in total GSL exposure on these properties was hampered by incomplete data; on four of the farms where disease had not been observed the bulb/crown data was missing and on five of the 'case farms' upper leaf data was likewise missing.

The distributions of data from affected and non-affected properties, with both bulb/crown and upper leaf data excluded, are illustrated in Figure 36. This suggests that there was a marginally greater exposure on case farms. Controlling both for plant part and cultivar through MANOVA, case status was of marginal significance ($P = 0.100$).

Figure 36 A box plot showing the distributions of log_{10} transformed mean plant-part total glucosinolates by disease status (illness and deaths observed = Yes, no illness and/or deaths observed = No) for swede samples collected during the swede associated liver disease event in Southland and south Otago in winter/spring 2014.



7.2.3 Specific glucosinolates

7.2.3.1 Concentration in different varieties and plant parts

A total of 21 GSLs were identified and a noteworthy result was the reported difference between the concentration of progoitrin and the rest. As illustrated in Figure 37, the concentration of progoitrin (mean = 24.8 µmol/g dry wt.) is of the order of 10 to 50 times the other GSLs[q] detected.

The individual plant part results, by HT® and not HT®, are presented in Figure 38. Interestingly, there appeared little difference between some plant parts in their individual GSL profiles. There were differences between the swede types and this was confirmed by statistical analyses (Table 15).

Table 15 Differences between plant parts, swede varieties and their interaction P values and means* (µmol/g dry matter) for swede samples collected during the swede associated liver disease event in Southland and south Otago in winter/spring 2014.

Glucosinolate	P Value			Mean				
	Plant Part	Variety	Plant part by variety	Lower Stem	Upper Stem	Flower	HT®	Not HT®
Progoitrin	0.317	0.011	0.605	23.5	31.9	34.9	38.5[a]	21.5[b]

Note: Means with no letters in common are significantly different at P<0.05.

Figure 37 Mean concentration (µmol/g dry matter) of all plant parts for all investigated glucosinolates for swede samples collected during the swede associated liver disease event

[q] See Appendix 2 for a coded list of all the glucosinolates which were investigated.

in Southland and south Otago in winter/spring 2014. See Appendix 2 for a table listing the codes (1 to 28) for each compound.

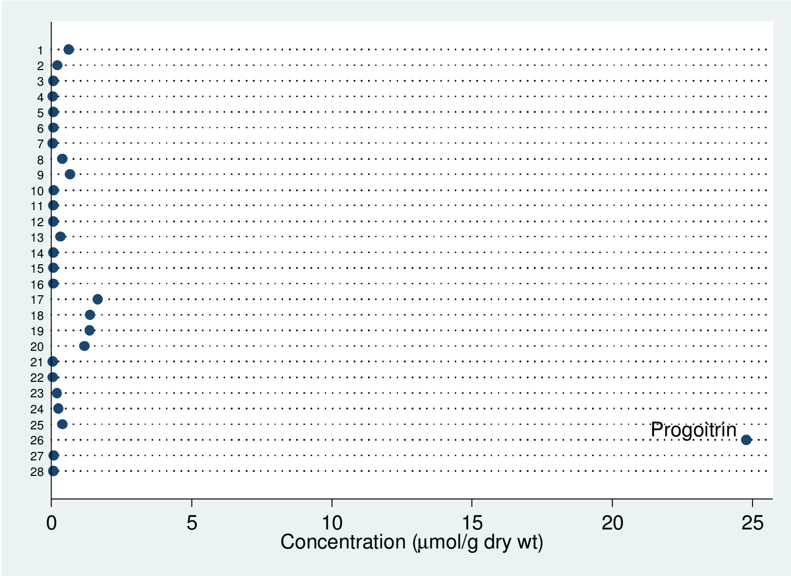
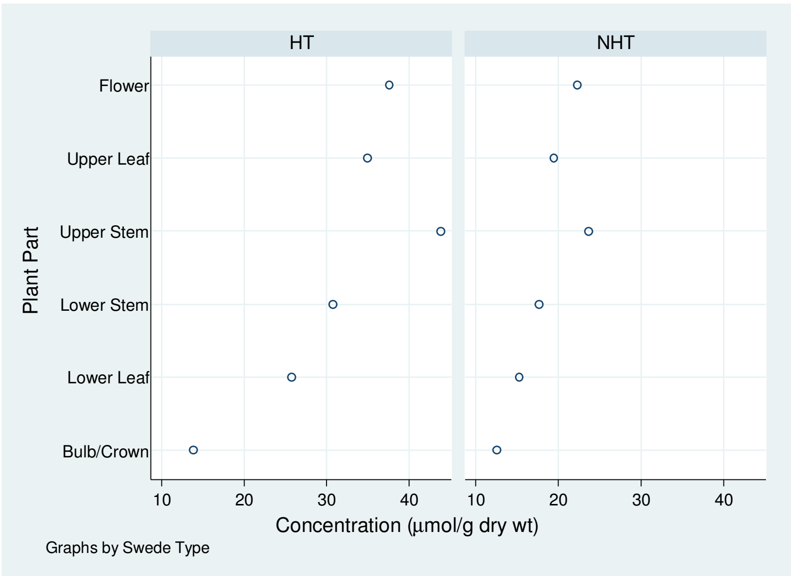


Figure 38 Progoitrin: stripplot showing the mean plant part results (µmol/g dry matter) by HT® and non-HT® swedes for swede samples collected during the swede associated liver disease event in Southland and south Otago in winter/spring 2014.



An intermediate cluster of compounds, with overall average concentrations in the range from 1 to 2 µmol/g, was also found (Figure 39). The results of the statistical analysis exploring differences between plant parts and swede types are listed in Table 16. In two of the four, there were significant ($P < 0.05$) differences between plant parts, and in two there were differences between swede varieties.

In one case, Gluconapin, there was a highly significant (P = 0.003) interaction term. The data points for all plant parts and for HT® versus non-HT® are plotted in Figure 40. This shows that there were differences between plant parts, but they were also different in HT® and non-HT® swedes; i.e. there is an interaction between plant part and swede type.

Table 16 Differences between plant parts, swede varieties and their interaction P values and means* (µmol/g dry matter) for swede samples collected during the swede associated liver disease event in Southland and south Otago in winter/spring 2014.

Glucosinolate	P Value			Mean				
	Plant Part	Variety	Plant part by variety	Lower Stem	Upper Stem	Flower	HT®	Not HT®
Gluconapin	<0.001	0.072	0.003	0.45^b	0.91^b	2.60^a	0.95	1.69
Gluconapoleiferin	0.885	0.835	0.997	1.31	2.49	1.53	1.99	1.56
Gluconasturtiin	0.003	0.005	0.606	1.87^a	0.86^b	0.68^b	1.55^a	0.72^b
Glucoraphanin	0.203	0.042	0.500	0.71	2.91	2.91	3.39^a	0.96^b

Figure 39 Mean concentration (µmol/g dry matter) of all plant parts for minor and moderate concentration glucosinolates for swede samples collected during the swede associated liver disease event in Southland and south Otago in winter/spring 2014.See Appendix 2 for a table listing the codes (1 to 28) for each compound.

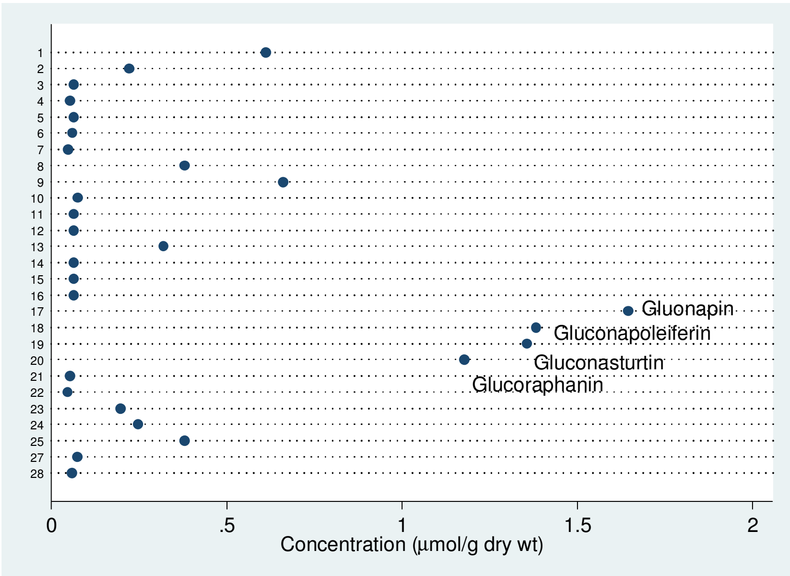
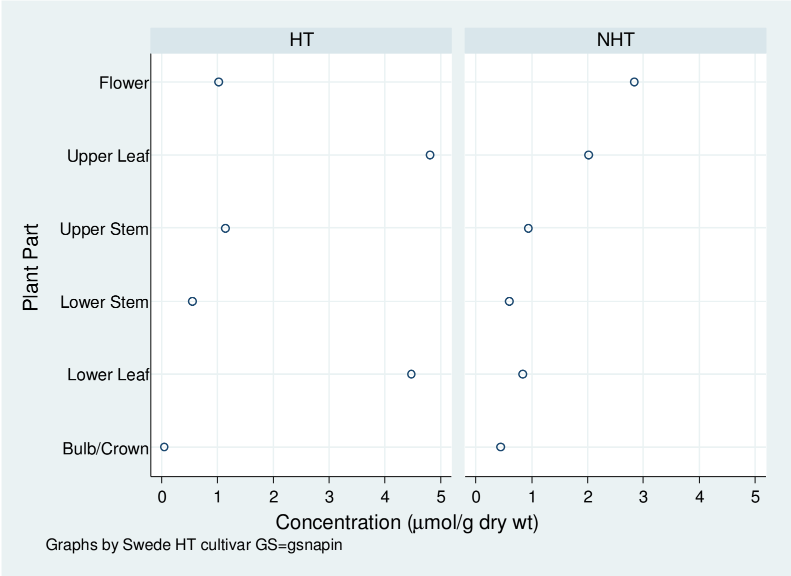


Figure 40 Gluconapin: stripplot showing the mean plant part results (µmol/g dry matter) by HT® and non-HT® swedes for samples collected during the swede associated liver disease event in Southland and south Otago in winter/spring 2014.



The balance of the results, either low concentration or less than the limit of detection, are listed in Table 17. In five (33%, 5/15) there were significant differences between plant parts; in three (20%, 3/15) there were differences between swede types; but there were no significant interactions between plant part and swede type.

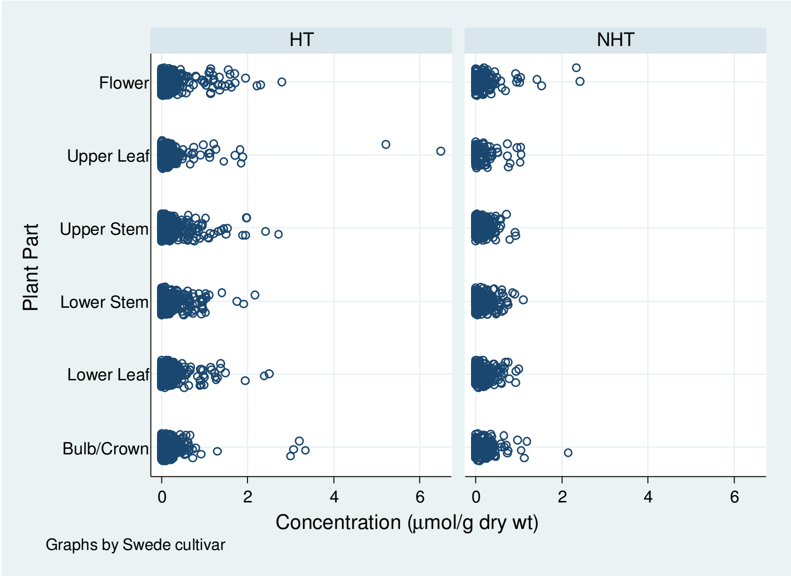
Table 17 Differences between plant parts, swede varieties and their interaction
P values and means* (µmol/g dry matter) for swede samples collected during the swede associated liver disease event in Southland and south Otago in winter/spring 2014.

Glucosinolate	P Value			Mean				
	Plant Part	Variety	Plant part by variety	Lower Stem	Upper Stem	Flower	HT®	Not HT®
Epiprogoitrin	0.257	0.029	0.797	0.53	0.72	0.89	0.92[a]	0.51[b]
Glucoalyssin	0.188	0.208	0.287	0.15	0.41	0.79	0.27	0.63
Glucoiberin	0.705	0.250	0.705	0.00	0.00	0.00	0.01	0.00
Glucosinolate A	0.997	0.281	0.997	0.00	0.00	0.00	0.00	0.00
Glucosinolate B	0.128	0.142	0.558	0.02	0.04	0.03	0.03	0.02
Glucobrassicanapin	0.363	0.835	0.660	0.11	0.21	0.52	0.26	0.31
Glucobrassicin	0.040	0.211	0.808	0.39[b]	0.71[ab]	1.06[a]	0.85	0.59
Glucoberteroin	0.038	0.123	0.222	0.16[a]	0.03[ab]	0.01[b]	0.03	0.10
Glucoerucin	0.182	0.139	0.440	0.45	0.35	0.09	0.42	0.18
Glucoraphenin	0.774	0.616	0.774	0.00	0.00	0.00	0.00	0.00
Glucotropaeolin	0.663	0.182	0.914	0.01	0.01	0.01	0.01	0.00
Hydroxyglucobrassicin	<0.001	0.006	0.258	0.09[b]	0.31[a]	0.31[a]	0.30[a]	0.17[b]
Methoxyglucobrassicin	<0.001	0.992	0.983	0.54[a]	0.21[b]	0.13[b]	0.29	0.29
Neoglucobrassicin	0.082	0.715	0.872	0.60	0.26	0.21	0.38	0.33
Sinalbin	0.957	0.465	0.935	0.02	0.04	0.03	0.05	0.01
Sinigrin	0.005	0.004	0.847	0.03[b]	0.04[b]	0.09[a]	0.07[a]	0.03[b]

Note: Means with no letters in common are significantly different at P<0.05.

The data points for these 'minor' compounds, at least in terms of concentration, are plotted in Figure 41. This indicates that although the majority of points were very low, there were sporadic moderate values (i.e. 1 to 3 µmol/g) and a few even higher, especially in HT® swedes and in flowers, upper stems and upper leaves.

Figure 41 Minor gluconsinolates: stripplot showing the individual plant part results (µmol/g dry matter) by non-HT® and HT® swedes for samples collected during the swede associated liver disease event in Southland and south Otago in winter/spring 2014.



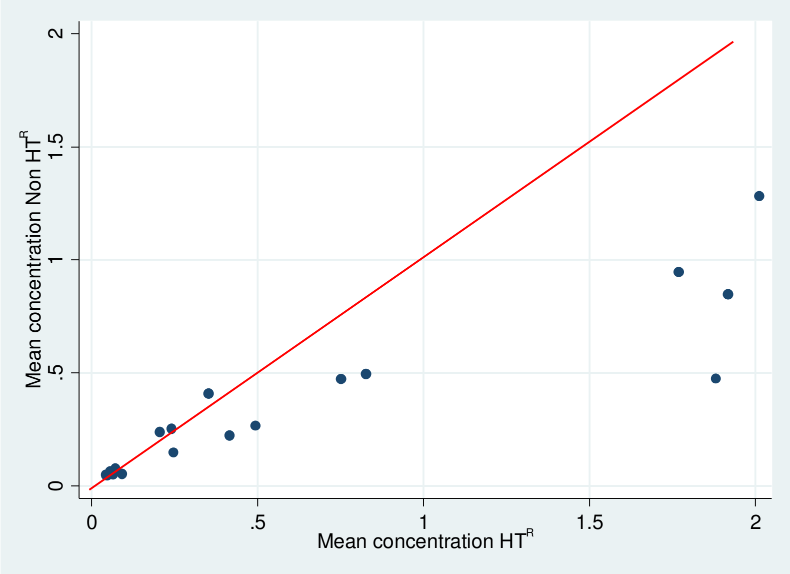
7.2.3.2 Glucosinolate composition of swedes

A series of analyses were also undertaken to determine if the 'glucosinolate composition' varied in a significant manner between varieties, plant parts, farms and samples. In other words, focus was on the combination of all 21 compounds that had been identified and whether or not these could be grouped under the various determinants of interest.

An overview of some results of this investigation is presented in Figure 42. This is a scattergram of the mean concentration of each GSLs[r], adjusted for the unbalanced nature of the data, with HT® values on the x axis and non-HT® values on the y axis. Among the low concentration compounds (< 0.5 µmol/g) there was little difference between the two swede cultivars. However, with the higher concentration compounds, there was a consistent pattern of a higher concentration in the HT® types.

[r] with the exception of progoitrin

Figure 42 Scattergram showing the adjusted mean concentration (µmol/g dry weight) of each glucosinolate in HT® (x axis) and non-HT® cultivars (y axis) from swede samples collected during the swede associated liver disease event in Southland and south Otago in winter/spring 2014.



7.2.4 Quality control aspects of analyses of swedes for glucosinolates

An objective of the investigation of GSLs in swedes was to assess the reliability of the process; i.e. from the field through to laboratory analysis. The literature suggests that degradation of GSLs starts as soon as the plant is damaged due to the release of local enzymes. Therefore there appears to be a potential for wide variation in outcome, and a monitoring system for 'swede toxicity' might not be practicable.

To test this, plant samples were duplicated and in addition, a series of replicate analyses were conducted by the laboratory.

The results of the investigation into the reliability of the process indicated that:

- For all GSLs, the variation between laboratory replicates was substantially lower than variation between duplicate samples
- The variation between laboratory replicates (as measured by the coefficient of variation ['CV']) was below 10% (3.7% to 7.8%) for all but one glucosinolate; glucoalyssin where the CV was 13.8%. This was a satisfactory result and, together with the validation work carried out by the analytical laboratory, indicates that the method was fit-for-purpose
- The variation between duplicate samples (also as measured by the coefficient of variation) was between 14% and 50%, with half below 25%. This was somewhat less satisfactory and a review of the methodology for collecting material from the field to reduce this variance should be considered.

Overall, the analytical method was deemed fit for purpose; to assess the potential toxicity of a swede crop; or the individual GSL profiles.

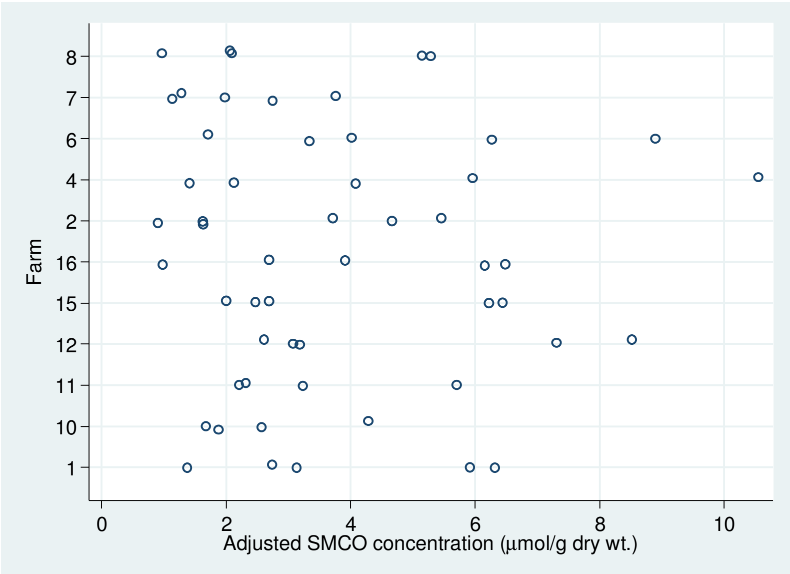
7.2.5 S-methyl-L-cysteine sulphoxide (SMCO) concentration

As described in Section 4, when some derivatives of SMCO are absorbed, they will oxidise haemoglobin in red blood cells. This leads to higher rates of red blood cell destruction in the spleen. High SMCO intakes can result in clinically significant outcomes, so-called 'Heinz body anaemia'.

In the cross-sectional survey of sick and apparently healthy cows (Section 5), there was no evidence of widespread anaemia. However, since on some farms there were sporadic cases SMCO was included in the analysis suite of putative pre-toxins of swedes, to confirm that intakes were less than that considered to cause toxic effects.

Swede material, as described above (Section 7.1) from 11 farms was analysed for SMCO as one of the analytes in the analysis suite. The mean result for each plant part, adjusted for unbalanced data, for each farm is presented in Figure 43. On all farms most data points lie between 1 and 7 µmol/g dry weight. The results confirm that SMCO concentrations were low and should not have caused toxicity issues for animals grazing swedes[9, 10] (Table 18).

Figure 43 Strip plot showing mean adjusted SMCO concentration (µmol/g dry weight) for each plant part from swede samples collected during the swede associated liver disease event in Southland and south Otago in winter/spring 2014. The data is adjusted for unbalanced data.



The mean concentration for each plant part, with the minimum and maximum, are listed in Table 18. Box plots of the individual data points, by plant part and by plant part and swede cultivar (HT® and non-HT®) are presented in Figures 44 and 45. This suggests that there are differences between plant parts but not between cultivar.

The results of the statistical analysis of this data were as follows:

- significant differences (P=0.002) between plant parts (flower and upper stem were higher than lower stem)
- no differences (P=0.494) between HT® and non-HT® swedes
- no interactions between cultivar and plant parts

However the results should be treated with some caution as SMCO has quite a different chemical structure to the GSLs. As Hill Laboratories was unable to source an analytical reference standard for SMCO the data were reported as “semi-quantitative”. It is suggested that before further SMCO testing is undertaken an inter-laboratory test should be completed to comparison the SMCO data. Hill Laboratories support this approach.

Table 18 Mean concentration of SMCO (µmol/g dry matter) and minimum and maximum by plant part and by swede variety for swede samples collected during the swede associated liver disease event in Southland and south Otago in winter/spring 2014.

Variety	Plant Part	Mean	Minimum	Maximum
All	Bulb/Crown	2.69	1.57	3.26
	Lower Leaf	1.56	0.87	2.62
	Lower Stem	2.59	1.54	4.10
	Upper Stem	6.15	2.69	10.58
	Upper Leaf	2.87	1.08	3.96
	Flower	6.02	3.79	8.47
HT®	Bulb/Crown	2.56	1.57	3.24
	Lower Leaf	1.46	0.87	2.62
	Lower Stem	2.79	1.54	4.10
	Upper Stem	6.59	2.69	10.58
	Upper Leaf	3.13	1.08	3.96
	Flower	6.10	3.79	8.47
Other (not HT®)	Bulb/Crown	2.96	2.66	3.26
	Lower Leaf	1.73	1.04	2.35
	Lower Stem	2.25	1.83	2.77
	Upper Stem	5.39	4.25	6.54
	Upper Leaf	2.36	2.17	2.54
	Flower	5.76	5.36	6.15

Figure 44 Box plots showing the SMCO concentration (µmol/g dry weight) in different plant parts from swede samples collected during the swede associated liver disease event in Southland and south Otago in winter/spring 2014.

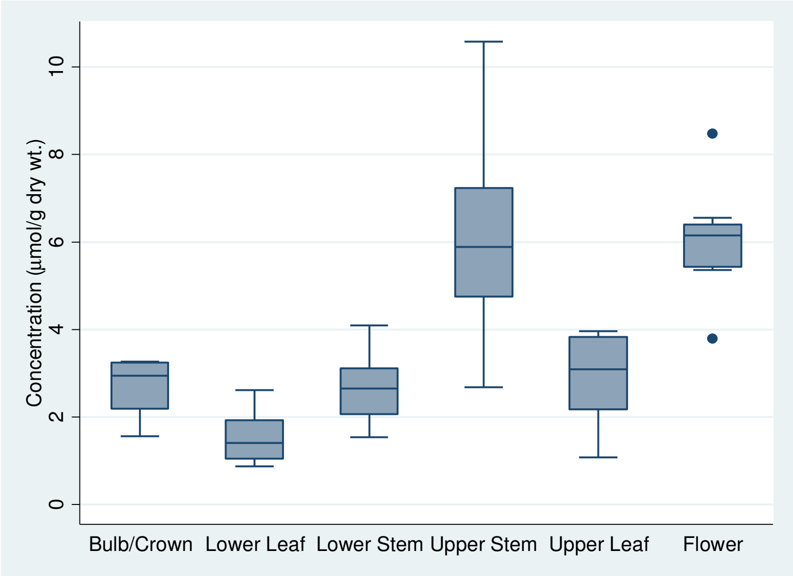
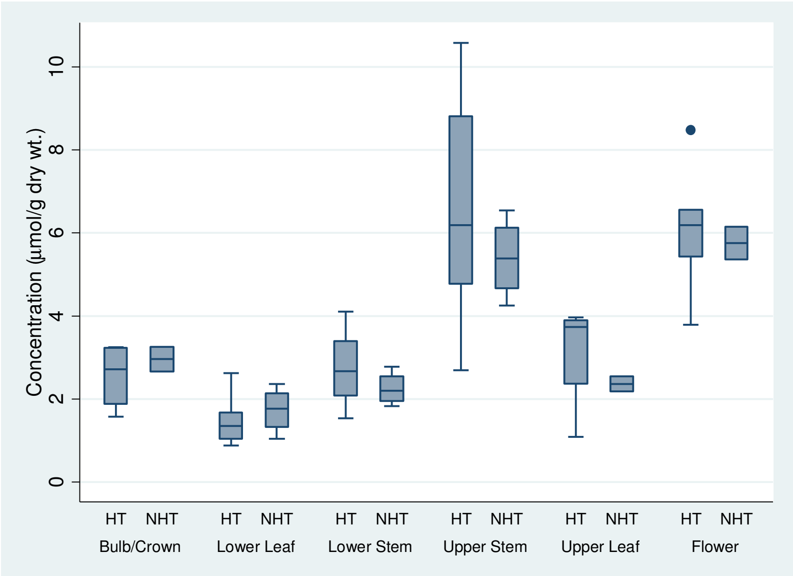


Figure 45 Box plots showing the SMCO concentration (µmol/g dry weight) in different plant parts by swede cultivar (HT® and non-HT®) from swede samples collected during the swede associated liver disease event in Southland and south Otago in winter/spring 2014.



8 Concluding remarks

As information about the problems that were occurring in dairy cattle grazing swedes in Southland and South Otago was assembled, it became increasingly clear that this was not a straightforward toxicosis. For this reason, a multi-facet investigation was launched by DairyNZ. It is important to remember that the activities were exploratory in nature with the aim of providing advice to farmers and their supporting advisers, to manage potential risks and try to avoid negative effects in the future.

The retrospective study that was undertaken was very challenging; and by its very nature prone to various types of bias, for example, recalling detailed information. The analysis was handicapped by missing data. There were also some difficulties assigning herds to ‘affected’ and ‘not-affected’ categories and thus so-called ‘selection bias’ may be present. Thus, one needs to take care when evaluating the results of this work.

That a new cultivar of swedes, ‘Herbicide Tolerant®’ (HT®), had fairly recently become available and was being used widely also presented some difficulties for the investigators. HT® swede crops were common on milking platforms because of historical weed issues from previous brassica crops. Many farmers would not have been able to use brassica crops on the milking platform (i.e. available for feeding in late pregnancy, calving and early lactation cows) because they would have been less able to control the weeds in non- HT® swede crops.

Clearly, it was essential that the effect of the new HT® swede should be included. However, there was considerable comment and inference that HT® swede was the cause of the adverse animal health and an unsubstantiated opinion that it was a ‘new disease’ caused by HT® swedes. This may have influenced responses in the survey.

One of the important initial questions that needed answering was ‘why were the animals ill?’.

One commonly reported condition, photosensitivity, drawing on parallels with facial eczema, pointed in the direction of liver disease. However, in many cases the symptoms were nondescript (ill-thrift, poor weight gains, metabolic problems etc.). Looking back, one would say that having a full pathological examination of only one animal was inadequate. However, information was being received from veterinary practitioners who had conducted a number of necropsies[3]. That toxic damage to the liver was the primary insult was borne out by the blood test results, in particular the elevated GGT levels and to a lesser degree the GLDH levels. But this was confusing as well, as 50% of all the animals tested, including many showing no apparent clinical signs of disease, had GGT levels greater than the ‘normal’ upper limit.

During the wintering period, in most herds only a small number of animals were affected. During late pregnancy, calving and early lactation, the situation was much worse, despite the time on swede crops being less. Clearly, resolving why this occurred is important. As all of the crops were sown at around the same time (November-December 2013), the maturity of the swede crop appears to have been a key determinant. The results of the plant analyses are in accord with this.

Another possible explanation or contributing factor for the difference in incidence during wintering and during/after calving is the physiological status of the animals. While there are no data supporting this as a major risk, it is a period when cows are metabolically challenged and could be expected to be less able to cope with toxins arising from high concentrations of total GSLs in their diet and a cautious approach should be taken during this period.

The results of the extensive screen for risk factors associated with ‘case’ herds was disappointing, as other analyses suggest that there are important ‘farm’ factors. Clearly, the finding that feeding HT® swedes during late pregnancy, calving and early lactation is strongly associated with disease in herds is significant; and in particular as it is coupled with a high incidence rate. Assuming the case and control herds in this analysis are representative samples of all case and control herds (i.e. no selection bias) and that there is a causal association, the data indicates that HT® swedes were associated with around 90% of disease over the period of late pregnancy, calving and early lactation. However, if selection bias is, unintentionally present, with more focus on HT® swedes, then the percentage may be overestimated.

The supplementary investigation of risk factors also yielded evidence that feeding swedes in general is associated with disease over wintering. The herds in this study were not selected with reference to wintering on other crops, and thus this finding should be viewed with care.

The analysis of GSLs in swedes uncovered another layer of complexity. There is good evidence that the concentration of these chemicals is affected by both intrinsic (especially genotype) and extrinsic (farm, weather etc.) factors. There is also the question of how important each ‘pre-toxin’ is. For example, progoitrin makes up a sizable proportion of the total GSLs but, as the name suggests, the derivatives are considered to affect thyroid function rather than cause liver damage. Until the relative importance of the different GSLs is defined in terms of breakdown products to potentially toxic compounds when consumed and digested, the total GSLs concentration is indicative only of the potential for causing adverse health events in livestock.

Monitoring profiles for individual GSLs by plant breeders when they select new varieties would determine if the individual GSLs profiles have changed during the development of the variety. In addition, monitoring the individual GSL concentrations through the swedes life cycle could be very useful for providing advice to farmers, particularly to understand the relative GSL concentrations at critical times when farmers may be feeding swedes. For example in autumn, before the first frosts, when grazing animals might eat more leaves than bulbs as the bulbs are hard and difficult to eat.

The now common practice of feeding swedes during calving and early lactation, in DairyNZ’s opinion, is a major contributory factor to the incidence of disease, as the practice increases the risk of feeding crop with higher levels of GSLs (i.e. feeding crop in the reproductive growth phase). This is further exacerbated by the use of HT® swede on the milking platform. Over the last one or two decades, swedes have moved from being a purely wintering crop to one now used by many farmers to fill the feed deficit on the milking platform in late winter. As swede crops for both scenarios are sown at the same time, during calving and early lactation cows will be exposed to a more mature crop, and thus the GSL intake will be higher. This was, most likely, exacerbated by crops reaching maturity more quickly in 2014. There was an unusually warm winter, higher rainfall and fewer frosts and many swede crops in early August 2014 had ‘bolted’, looking more like crops seen much later in the season. As presented in Figure 46, the differences between many of the crops seen in winter 2014 and those in 2015 are very marked.

Figure 46 Typical ‘bolted’ swede seen commonly in 2014 (left) and a ‘normal’ swede observed in 2015 (right).

Considering the results of the work that was undertaken by DairyNZ, two general items standout:

- The complexity of this outbreak of disease, involving animal, plant, farm and, most likely, weather factors together, with changes over time
- The evidence that in many apparently healthy cattle grazing swedes there is some liver damage.

It appears as if the causal factors came together in such a way during winter and spring in 2014 and this contributed to the clinical presentation of a risk associated with feeding brassicas, rather than this being a new disease outbreak.

Accepting the limitation of the retrospective investigation, one might consider setting up more controlled prospective studies to test key hypotheses. Unfortunately, the many factors that can lead to adverse animal health effects when grazing swedes (i.e. swede varieties and growth, which in turn are influenced by paddock effects, disease, climate and farm management practices, combined with the animal factors, and individual animal behaviours) which were present in the winter of 2014 would be impossible to replicate.

9 Recommendations

As a result of the work carried out over the last year, DairyNZ recommends that farmers do not feed HT® swedes on the milking platform in late August/early September (i.e. late pregnancy, early lactation). This is when many of the known factors (warmer temperatures, new leaf growth, 'bolting'/stem elongation) that lead to ill-heath and potential cow deaths can rapidly combine.

This recommendation is based on the following factors:

- As soon as the weather begins to warm the swedes will begin to enter the reproductive growth phase. HT® swedes have a higher concentration of total GSLs in the plant parts where re-growth occurs. Beware of other leafy swede varieties as well.
- Heavily pregnant, springing and milking cows grazing swedes during winter and early spring, may be under pressure from toxins generated through consuming GSLs through that period
- The cows are metabolically challenged due to late pregnancy and early lactation physiological changes and less able to cope with toxins arising from high concentrations of total GSLs in their diet.

The following actions are also recommended:

- Do not feed swede crops in their reproductive growth phase. This is recognisable when the swede's stem elongates, new growth appears and the swede plant develops flowers and a seed head (referred to as 'bolting').
- In autumn, before the first frosts, be cautious when grazing animals on swede crops as they might eat more leaves than bulbs as the bulbs are hard and difficult to eat.
- At any time during the season, be cautious when grazing animals on swede crops with a high leaf-to-bulb ratio, as cows may preferentially graze leaf.
- Observe the physical characteristics of the crop being fed, monitor the health of cows and adjust their feed management if incidences of ill-health are observed.
- Refer to DairyNZ Advisory #11 for more information around feeding management (Appendix 4).
- Follow PGG Wrightson Seeds endorsements (as at 30 November 2015) regarding HT® swedes and their use.

The most effective means of managing risk in the future could be:

For farmers to:

- Simplify their winter feeding systems to minimise the transitioning requirements for animals as they change feeds (i.e. pasture to crop; crop to crop; crop to pasture) (DairyNZ Advisory #12 Appendix 5)
- To use farm management practices that reduce the potential for an individual animal behaviour which deviates from herd behaviour (i.e. dominant cows grazing only leaves)
- Monitor a random selection of animals to ascertain if blood profiles are normal or whether the animal is under metabolic stress.

For plant breeders to:

- Monitor the individual GSL concentrations through the swedes life cycle to understand the relative GSL concentrations at critical times when farmers may be feeding swedes.
- Monitor new varieties of swedes for individual GSL concentrations to ascertain if the profile has changed with the new variety.

Researchers to:

- Complete the toxicology research into the toxic effects of individual GSLs being undertaken by Mark Collett, (Massey University and PGG Wrightson Seeds)
- Complete the blood monitoring that was initiated during 2015 to define a range of expected concentrations for liver enzymes as animals' transition onto and remain on crop.

10 Appendices

10.1 Appendix 1: Members of the Swede Working Group (SWG)

An invitation was made to interested parties including industry stakeholder, veterinary practices and plant breeders. The Table below summarises those who accepted the invitation and were members of the SWG. The table shows the organisation each person represented and their role.

Table 19: shows a summary of the SWG members, the organisation each person represented and their role. Primary contacts are highlighted.

Name	Organisation and role
Richard Kyte	Chairman SWG; and DairyNZ Regional Leader
Dawn Dalley	DairyNZ Senior Scientist with particular expertise in whole farm systems and winter management in the South Island
Anna Irwin	DairyNZ Animal Husbandry Extension Specialist with specialist with a Bachelor of Veterinary Science
Mark Bryan	VetSouth Managing Director, practicing veterinarian and epidemiologist
Teressa Skevington	Otautau Vets, Practicing Veterinarian
Allan Baird	Federated Farmers, Southland Dairy Chair
Russell MacPherson	Federated Farmers, Provincial President
Tanith Robb	Federated Farmers, Policy
Paul McCauley	Beef and Lamb New Zealand: Regional Extension Manager
David Green	PGG Wrightson Seeds, General Manager New Zealand Seeds
Charlotte Westwood	PGG Wrightson Seeds, Veterinarian
Andrew Dumbleton	PGG Wrightson Seeds, Product Development Manager – Seeds
Mark Collett	Massey University. Veterinary Pathologist
Glen Bradbury	Ministry of Primary Industries (MPI) Manager Agricultural Compounds and Veterinary Medicines
Awilda Baoumgren	MPI, Veterinarian
Jenny Weston	New Zealand Veterinary Association (NZVA) representative.
Morgan Greene	Northern Southern Veterinary Services, practicing veterinarian

As appropriate other people were invited to attend and contribute to the meeting as depending on the agenda items proposed.

The SWG group is expected to be disestablished with the review, feedback and release of this final phase – this report.

10.2 Appendix 2: Glucosinolates investigated with codes

Glucosinolate	Code
Epiprogoitrin	1
Glucoalyssin	2
Glucoibarin	3
Glucoiberin	4
Glucoiberverin	5
Glucosinolate A	6
Glucosinolate B	7
Glucobrassicanapin	8
Glucobrassicin	9
Glucoberteroin	10
Glucocheirolin	11
Glucodehydroerucin	12
Glucoerucin	13
Glucohirsutin	14
Glucohesperin	15
Glucolesquerellin	16
Gluconapin	17
Gluconapoleiferin	18
Gluconasturtiin	19
Glucoraphanin	20
Glucoraphenin	21
Glucotropaeolin	22
Hydroxyglucobrassicin	23
Methoxyglucobrassicin	24
Neoglucobrassicin	25
Progoitrin	26
Sinalbin	27
Sinigrin	28

10.3 Appendix 3: Climate data for 2014

A summary of the climatic conditions for air temperature during winter and spring 2014 expressed in terms of its departure from the published values for ten year average data are shown in Figure 47. The pattern of monthly rainfall totals for 2014, together with the ten year average data are shown in Figure 48.

Figure 47 Departure from ten year average air temperate for April 2014 to September 2014 (Source NIWA; http://www.niwa.co.nz/climate/nzcu Accessed 13 May 2015).

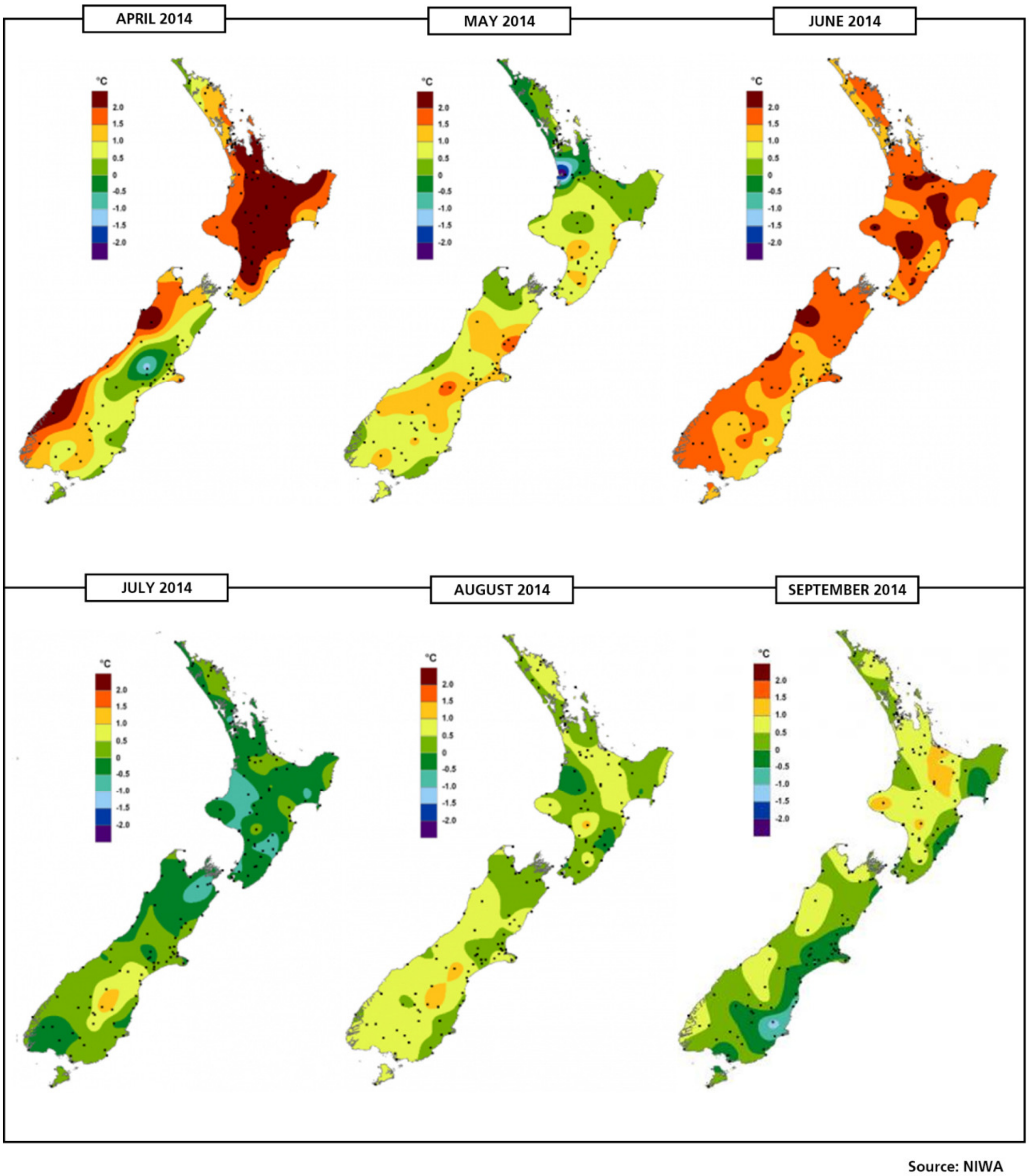
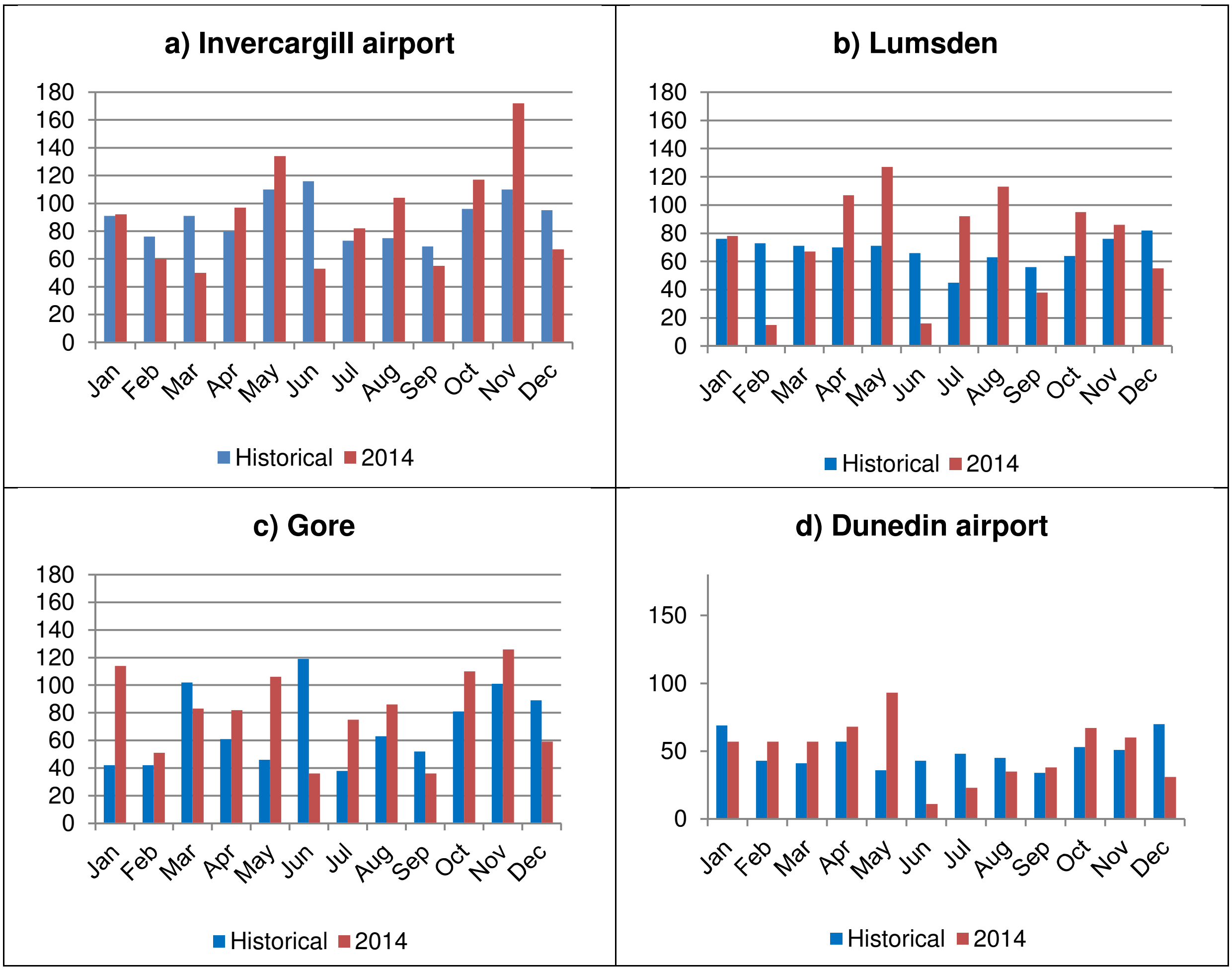


Figure 48 Monthly rainfall for 2014 and the ten year average of historical data for a) Invercargill airport, b) Lumsden, c) Gore and d) Dunedin airport (Source NIWA; http://www.metservice.com/towns-cities/dunedin Accessed 12 May 2015).



Swede advisory update#11

3 September 2015

Preliminary plant analysis findings

1. Plant samples were collected in the third week of September 2014 from farms where health issues had been experienced with HT-swedes (7 farms) and non HT-swede varieties (4 farms).

Physical characteristics of swedes

The plant samples looked unusual, with many of the swedes having high leaf to bulb ratios and "elongated" stems up to 1 m tall (bolted). The difference between the physical characteristics is illustrated below with August 2015 considered to be more 'normal'.

Warmer temperatures and fewer frost days in 2014 caused the swedes to keep growing, with crops going into their reproductive stage in August.

Inspection of the climate data supports the view that climatic conditions in winter and spring of 2014 differed from published ten year average climate data. Air temperatures from April through June, as well as in August, were generally warmer than normal, at times and in places by as much as 1.5°C (see Temperature graphs attached). In addition, rainfall was generally higher.

Figure 1: Comparison of swede physical appearance: 2014 and 2 0 1 5

September 2014

August 2015

2. *Plant parts analysed*

DairyNZ staff collected swede samples, dissected plants as quickly as possible and froze the samples in liquid nitrogen (-80°C) to stop any spoiling of the plant material and break down of the glucosinolates (GSLs), the naturally occurring compounds in brassicas that have been linked to cow health problems.

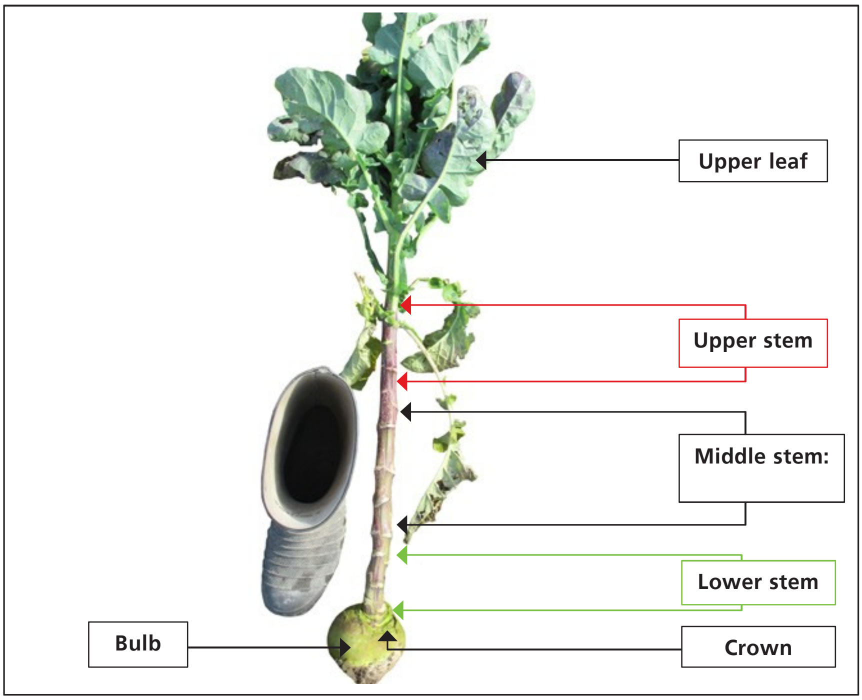
Samples were dissected into up to 6 plant parts so that each section could be analysed separately. Up to

150 plant parts were analysed from 3 swede varieties and across 11 different farms.

Due to the difficulty working with liquid nitrogen in the field, DairyNZ staff focused on the plant parts, which according to scientific evidence, were most likely to be causing the i s s u e .

Figure 2: DairyNZ staff in-field with liquid nitrogen

Figure 3: Plant parts analysed



3. *Preliminary findings from plant analysis*

The plant samples were tested for 30 different GSLs of which 23 were selected for further analysis – the other 7 were below the limit of detection.

The most significant findings from the samples analysed are:

1. Total GSL concentrations (µmoles per dry gram) are generally higher in the HT swede than in the non-HT swede varieties (Figures 4 & 5).

While there is not much difference between HT and non-HT swedes for bulb/crown, GSL concentrations in the other plant parts are generally higher for HT-swedes, with a pronounced difference in the upper leaf and upper stem.

Figure 4: Total GSL concentration by plant part by swede variety

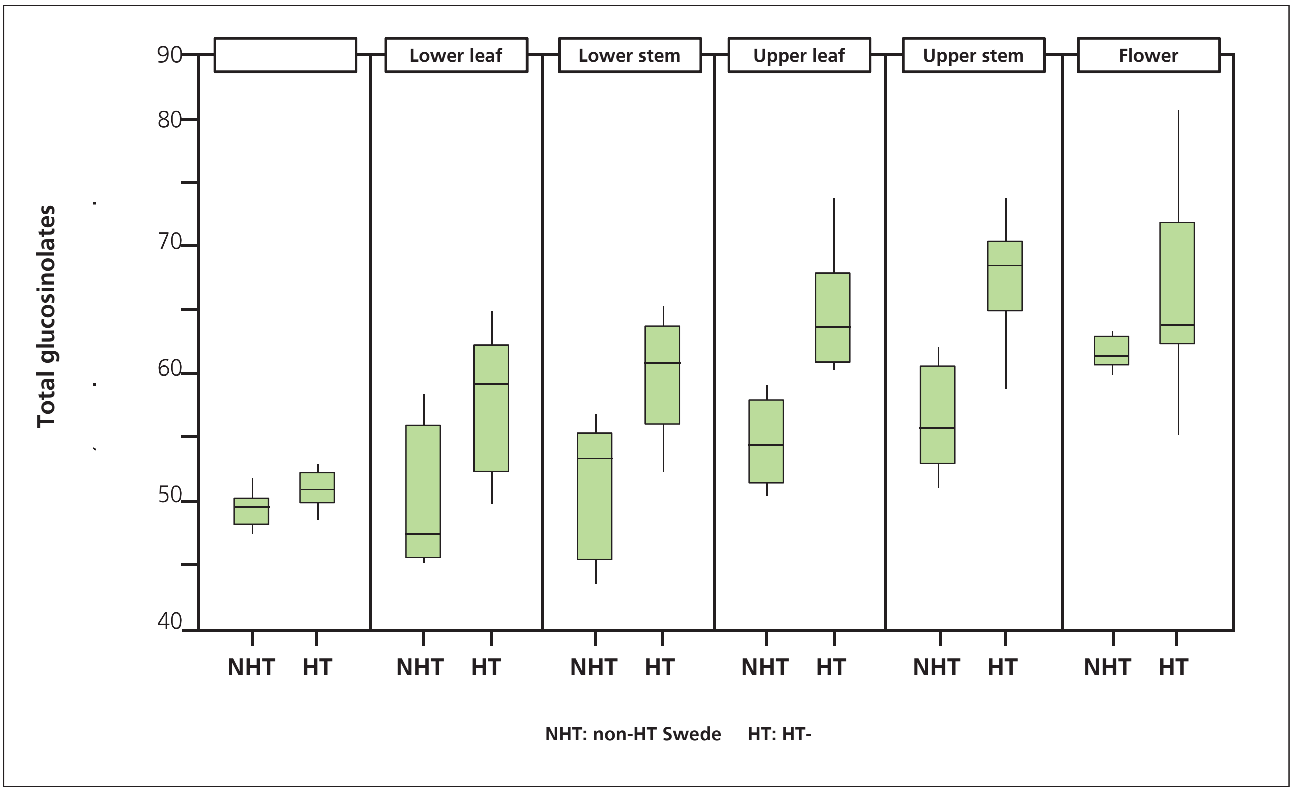
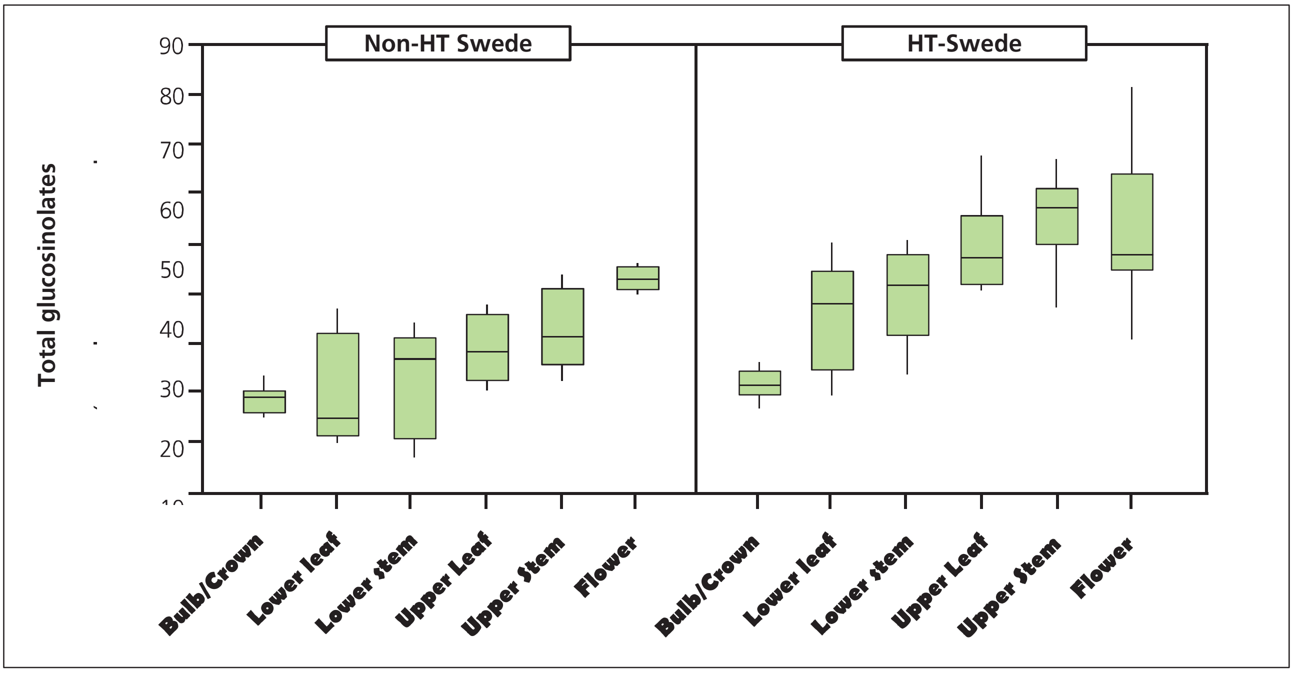


Figure 4 highlights the differences in GSL concentration for the plant parts analysed by swede variety

Figure 5 shows that GSL concentrations increase further up the plant, with concentrations higher in HT-swedes than in non HT-swedes.

Figure 5: Total GSL concentration by swede variety by plant part



2. No single GSL stands out as significantly different between plant variety and plant part.
   - Different parts of the plants included different levels of individual GSLs
   - The concentrations of individual GSLs varied between plant parts.

   This is supported by scientific literature that indicates that different genetic backgrounds can produce large differences in individual GSL concentrations.

   The literature also indicates that adverse effects in animals have generally been correlated to the amount of total GSLs in the diet, despite the fact that individual GSLs vary in their toxicity.

   For results of individual GSL by swede variety and by plant part see Individual GSLs charts attached.

3. In scientific literature it is reported that total GSL concentrations above 17µmol/g of GSLs consumed will reduce feed intake and milk production. Above 31µmol/g of GSLs consumed cows will show signs of toxic effects. For more information see:

   i. Waldern, D.E., 1973. Rapeseed meal versus soybean meal as the only protein supplement for lactating cows fed corn silage roughage. Can. J. Anim. Sci. 53, 107–112.
   ii. Ingalls, J.R., Sharma, H.R., 1975. Feeding of Bronowski, Span and commercial rapeseed meal with or without addition of molasses or mustard in ration of lactating cows. Can. J. Anim. Sci. 55, 721–729.
   iii. Laarveld, B., Brockman, R.P., Christensen, D.A., 1981c. The goitrogenic potential of Tower and Midas rapeseed meal in dairy cow determined by thyrotropin-releasing hormone test. Can. J. Anim. Sci. 61, 141–149.
   iv. Ahlin, K.A, Emanuelson, M. and Wiktorson, H. 1994. Rape seed products from double-low cultivars as feed for dairy cows: effects of long term feeding on thyroid function, fertility and animal health. Acta Veterinaria Scandinavia. 35:37-53.

The preliminary findings from the plant analysis confirms the hypothesis that the risk of ill-health and death in cows increases when total GSL concentrations increase as swedes enter the reproductive stage (elongated stem, new leaf, flowers and seed heads).

Advice to farmers

1. Feeding swedes in spring 2015

The new plant data supports the advice provided in Swede Advisory #10 June 2015.

Farmers should be very cautious when feeding swedes, in spring this year. Special care is needed with HT-swedes and other leafy varieties (e.g. Aparimu Gold, and Triumph), if/when warm air temperatures from northerly weather conditions, cause swedes to regrow and change quickly.

HT-swedes have higher concentrations of GSLs in “reproductive” plant parts, increasing the risk of ill-health for cows grazing swedes with elongated stems and the appearance of flowers. DairyNZ recommends extreme caution when grazing any swedes that have bolted.

DairyNZ recommends that farmers do not feed HT-swedes on the milking platform in late August/early September (late pregnancy, early lactation) when all the factors that lead to ill-heath and potential cow deaths (warmer temperatures, new leaf growth, bolting) can rapidly combine.

This recommendation is based on the following factors:

- As soon as the weather begins to warm the swedes will begin to enter the reproductive phase. HT-swedes have a higher concentration of total GSLs in the plant parts where re-growth occurs. Beware of other leafy swede varieties as well.
- Heavily pregnant, springing and milking cows grazing swedes during winter and early spring, may be under pressure from toxins generated through consuming GSLs through that period
- The cows are metabolically challenged due to late pregnancy and early lactation physiological changes and less able to cope with toxins arising from high concentrations of total GSLs in their diet.

Extreme caution is advised when swedes begin to regrow, especially HT-swedes and other leafy varieties.

It is essential that farmers observe the physical characteristics of the crop being fed, monitor the health of their cows and adjust their feed management if incidences of ill-health are observed.

For further information on the signs to look for and the actions to take see Swede Advisory #10.

2. Using HT-Swedes in your winter feeding programme

Should farmers choose to include HT-swedes in their winter feeding programme for 2016, or in future years, DairyNZ recommends that farmers use HT- swedes strategically.

We advise caution when starting to feed swedes in autumn as swedes that have not been frosted are likely to have lush, strong leaf growth. Should weather conditions change during winter where leaf growth and reproductive status are accelerated farmers should exercise caution and remain vigilant

New leaf growth in autumn swedes may also have higher total GSLs. Also as the bulbs are still hard and difficult for cows to eat they may prefer grazing leaves. After two or three frosts the swede bulbs are softer and easier to consume and the leaves die back and start to drop off. The cows are then more likely to consume a better ratio of bulb to leaf.

DairyNZ recommends that farmers do not feed HT-swedes on the milking platform in spring (late pregnancy, early lactation) when all the factors that lead to ill-heath and potential cow deaths (warmer temperatures, new leaf growth, bolting) can rapidly combine.

3. PGG Wrightson Seeds advice

Refer to PGG Wrightson Seeds endorsements and advice if you are considering HT-swedes as part of your wintering programme.

“PGG Wrightson Seeds recommends the prudent approach is that HT Swede (HT-S57) should not be grazed by pregnant or lactating dairy cows.
This recommendation will be reviewed as more information becomes available from the scientific research being undertaken”.

What is happening next?

1. DairyNZ is still monitoring the health of a few cows this season. This is the first step in assessing how we can develop baseline monitoring for detecting early signs of ill-health in cows.
2. Farmer survey

   - DairyNZ will be conducting two confidential on-line surveys to a random selection of Southland/South Otago farmers.
   - The first survey will be on farmers' experiences with feeding crops during winter and spring and will be sent out during the first week of September 2015.
   - The second survey will be on farmers' experiences with feeding crops during late pregnancy and early lactation and will be sent out around the end of September/beginning of October 2015.

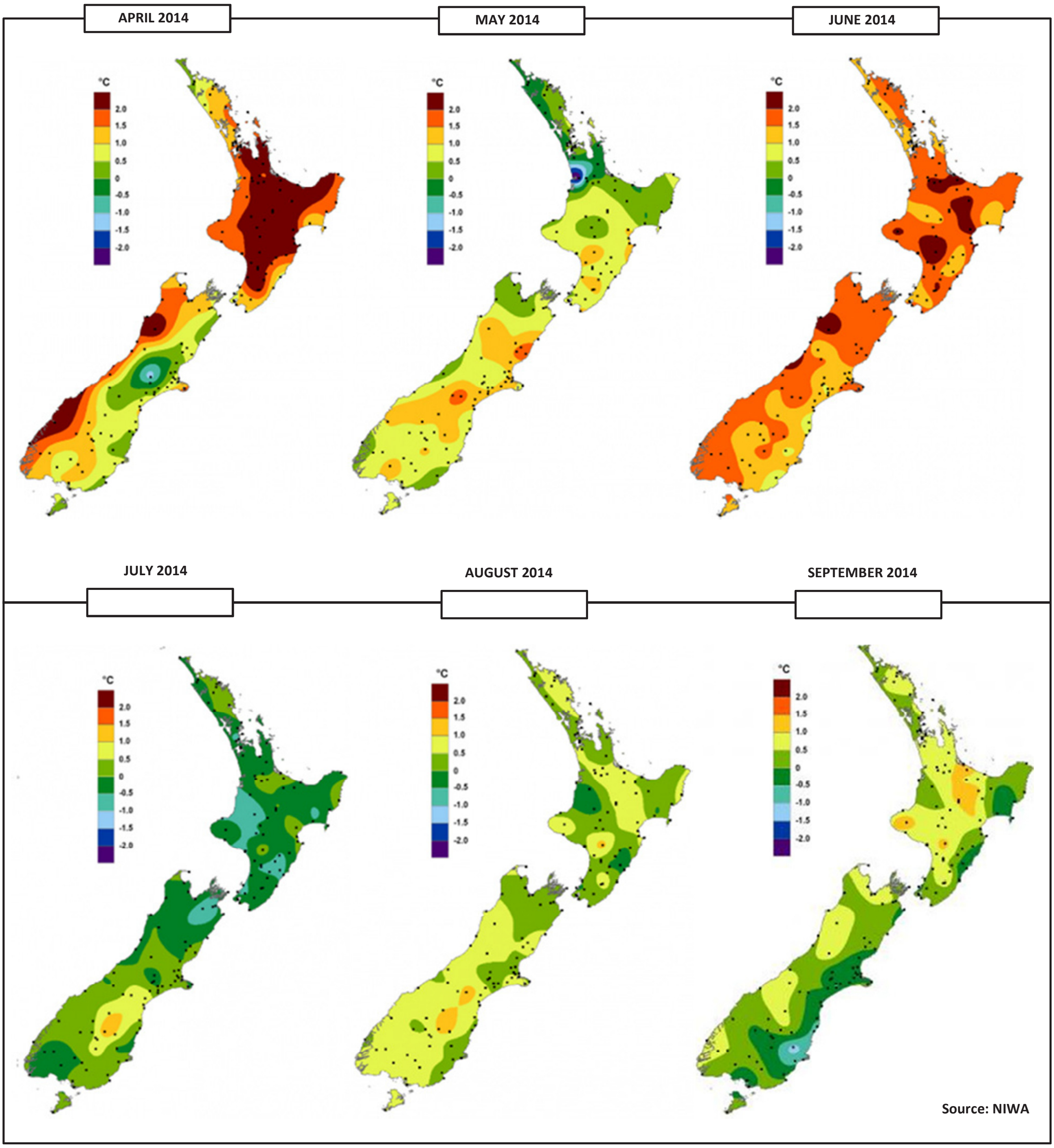
The information from these surveys will help DairyNZ to:

- Better target information to provide advice to farmers; and
- Identify future research requirements for managing crops.

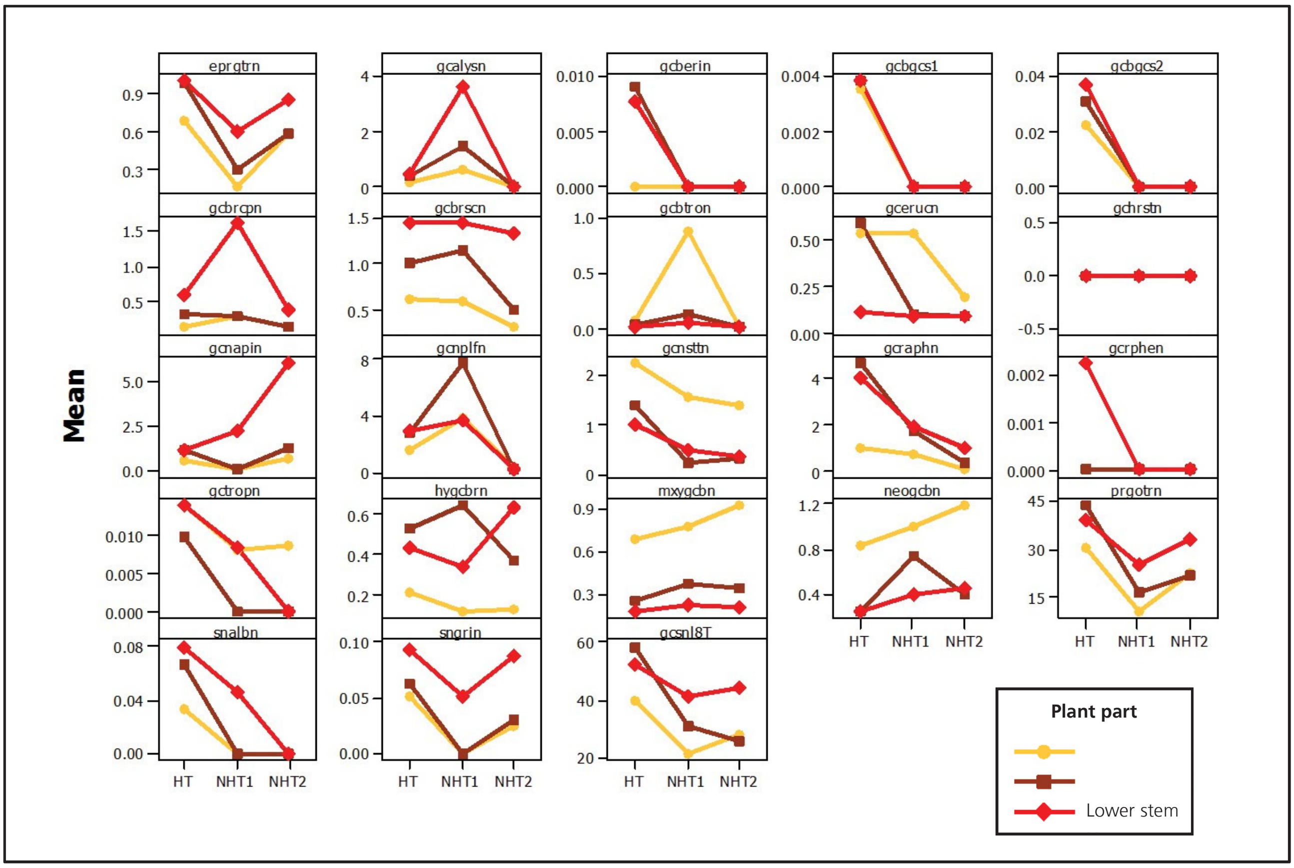
Prepared in consultation with the Southland Swede Working Group: Beef+Lamb, DairyNZ, Federated Farmers, PGG Wrightson Seeds, Ministry for Primary Industries, Rural Support Trust, New Zealand Veterinary Association and local veterinarians.

Temperature data

Departure from ten year average air temperature



Individual glucosinolate concentrations between varieties and plant parts



HT HT-swede **NHT** non-HT swede

Code	Name	Code	Name
eprgtrn	Epiprogoitrin	gcnsttn	Gluconasturtiin
gcalysn	Glucoalyssin	gcraphn	Glucoraphanin
gcberin	Glucoiberin	gcrphen	
gcbgcs1		gctropn	Glucotropaeolin
gcbgcs2		hygcbrn	Hydroxyglucobrassicin
gcbrcpn	Glucobrassicanapin	mxygcbn	Methoxyglucobrassicin
gcbrscn	Glucobrassicin	neogcbn	Neoglucobrassicin
gcbtron	Glucoberteroin	prgotrn	Progoitrin
gcerucn	Glucoerucin	snalbn	Sinalbin
gchrstn		sngrin	Sinigrin
gcnapin	Gluconapin	gcsnl8T	Total Glucosinolates
gcnplfn	Gluconapoleiferin		

Swede advisory update #12 24 September 2015

Preparing for Winter/Spring 2016

Preparation for winter and spring 2016 begins now with planning your winter feed budget and determining what your wintering system will look like. This is critical this year as some farmers look to winter more stock themselves.

Wintering in the Southland/South Otago region, where pasture growth rates are low most years from May until August, creates challenges for feeding dairy cows. Forage crops (brassica and fodder beet), which provide high yielding high quality feed that can be grazed, are essential feed sources in winter and as a supplement to pasture in early lactation and are critical for the success of dairying in the southern South Island.

There are many crop choices available, with each crop having its own merits. Brassicas, fodder beet and cereals are the main crops suitable for winter grazing, and there are several options within these groups. Conserved feeds such as silage, hay and baleage are also required to complement these crops.

DairyNZ recommends that you:

- Seek expert advice from your seed technical field rep, seed company or farm consultant when finalising your crop selection options to ensure the chosen feeds will achieve your wintering goals.
- Ensure you have plenty of supplementary feed (silage, baleage, straw, hay) to offer with all crops.
- Simplify your winter feeding system. Some diversity with crops is good to minimise the impact of a specific crop failure but minimise the number of different crops you grow to simplify your transitioning processes and winter feed allocation. Cows should be transitioned onto crops and between crop types and varieties. So the fewer crop types (e.g. kale vs swede) and varieties (e.g. Triumph vs Domain) of the same crop in your system the less transitioning required.
- Grow the same crop and variety on the milking platform that the cows will be offered during the winter (on support block, at grazier) to simplify transitioning and minimise risk. The exception is HT swede which should not be grown on the milking platform to be fed late winter/early spring.
- Work out the mobs that will be wintered and as far as possible match your paddock sizes and your crop type to the herd sizes so cows don't need to change crop type during the winter.
- Seek expert advice regarding the best way to manage and feed cows over winter if you are unsure.
- Consider how you will set up your wintering mobs with regards body condition score (BCS) and calving date. Ideally, initially setting up mobs based on BCS, to allow differential feeding of groups, then re-sorting into calving mobs later in the winter will provide the best opportunity to achieve BCS targets without excess feed input. If you can't change cows between mobs during winter, planning your autumn feeding, milking frequency and drying off strategy to minimise the BCS range at dry off is recommended.

- Consider the safest way to offer the crop if you choose to mix different crops in the same paddock (e.g. swedes and kale, turnips and moata). Sowing mixed seed rather than strip sowing individual crops reduces the risk of cows selecting a particular crop and eating only that type.
- Avoid planting different crops at alternate ends (or opposite sides) of the same paddock or allowing animals free access to different crop types on the same day (e.g. fodder beet & kale) to ensure all cows are eating a balanced diet.
- Select paddocks that will more easily enable cows to graze long narrow faces of crop.
- Consider the risks involved with each crop and the options for minimizing these risks, which includes having additional supplement available to increase the supplement to crop ratio if required to mitigate nutritional issues that may arise.
- Have 7-10 days of maintenance supplementary feed (70-100 kg DM/cow) available, in addition to your feed budget requirements, as part of your winter feeding programme. Even though times are tight financially having this feed for when unplanned events occur (snow, southerly storms, crop nutrition issues) will give you the space to plan and seek additional inputs if required.
- Speak with your grazier(s) about how they will be managing your cows, keeping in mind these recommendations.

A healthy well grown crop is a good investment.

Regardless of where crops are grown, it is critical to grow them well. For the same level of inputs, dry matter yield can vary significantly. A well-managed crop can produce double the yield of a poorly grown crop and requires only half the land area. In addition, healthy well grown crops provide the best nutritional value for animals. Anti-nutritional factors increase if the crop is under stress (lack of water, the presence of weeds, pests, plant disease, or poor soil fertility).
Anti-nutritional factors include nitrates, glucosinolates and SMCO. DairyNZ recommends that you consult with your seed supplier, seed company or consultant for the right agronomy advice for your crop. For more information on plant options as well as factors to consider and to discuss with your expert seed adviser to get relevant crop information (see Swede advisory #3 **dairynz.co.nz/swedes**).

Revise your feed budget often

It is important to review and revise your feed budget regularly during the season. We recommend that crop yields be completed at strategic intervals so that you can make adjustments to the plan and seek additional feed sources if required. We suggest:

February/March: assess how well the crop establishment and growth has gone, what the feed situation is like on the milking platform and how herd BCS is tracking. If things are off track at this point you still have time to make some changes.

May: finalise the winter feed budget and allocate mobs to crops

Mid July: revise your late winter/spring feed budget. Late crops may be carrying extra DM as a result of growth during winter and the areas remaining may differ from your plan depending on winter grazing conditions.

- *Swedes*

All swedes contain glucosinolates (GSLs) and it is generally accepted that swede leaves have higher levels of GSL's than swede bulbs. New leaf growth in all swedes may also have higher total GSL's than old leaves. The amount of total GSL consumed in the diet will impact on intake and growth rates of stock and determine the risk of toxic effects resulting in ill-health and potentially animal deaths.

The higher the proportion of swedes in the diet, the higher the risk of GSLs being consumed and the greater the risk of ill-health and potentially animals deaths.

It is therefore important to adopt management practices that minimise the risks, such as:

- Good transitioning onto the crop in early winter and between paddocks during winter (see DairyNZ Farmfact 1-75 Feeding winter brassica crops to dairy cows, **dairynz.co.nz/publications/farmfacts/farm-management**). Appropriate transitioning will minimise the risk of nutritional disorders from changes in the type of feed being offered and also any anti-nutritional factors that may be present.

- applying caution when starting to feed all swedes in autumn as swedes that have not been frosted are likely to have lush, strong leaf growth which could be high in anti-nutritional factors. Also as the bulbs are still hard and difficult for cows to eat they may prefer grazing leaves.
- Offering more supplement during transition if cows are slow to start eating the bulbs, rather than increasing the crop allocation to fill the gap, as offering more crop will just increase leaf intake.
- Always feed supplement (silage, baleage, hay, straw) before feeding crop so that cows are not hungry when they graze the crop.
- Feeding practices that encourage the consumption of both leaf and bulb by grazing long narrow faces.
- Visually assessing the crop for bolting stems, new leaves and reproductive development throughout the winter feeding period. If crops start to change during winter/early spring farmers should exercise caution and remain vigilant.
- Not feeding any swedes with elongated stems and reproductive tissue ("bolted" swedes).

It is also generally accepted that because crops are selected for various traits, such as DM yield and quality, the composition of individual glucosinolates in the swedes and the swede components vary.

HT Swedes

DairyNZ recommends that farmers

- Use HT-swedes strategically and follow the advice of PGG Wrightson Seeds not to feed HT swedes to pregnant or lactating cattle, if you choose to include HT-swedes in your winter feeding programme for 2016, or in future years. Survey results indicate the risk of ill health with HT swedes is higher late in the season when crops are more mature and cows are in late pregnancy or early lactation.
- Do not feed HT swedes to cattle in spring when warmer temperatures increase the risk of swedes 'bolting' (elongated stems, new leaves, seedheads, and flowers) which have higher concentrations of GSLs, the naturally occurring compounds in brassicas that have been linked to ill-health and animal deaths.
- Do not feed any "bolted swedes" from any swede variety. Consequently farmers can ill afford a lost crop due to an early spring or "bolted swedes".

DairyNZ also draws your attention to PGG Wrightson Seeds endorsements and advice if you are considering HT swedes as part of your winter programme.

"PGG Wrightson Seeds recommends the prudent approach is that HT Swede (HT-S57) should not be grazed by pregnant or lactating dairy cows. This recommendation will be reviewed as more information becomes available from the scientific research being undertaken".

Prepared in consultation with the Southland Swede Working Group: Beef+Lamb, DairyNZ, Federated Farmers, PGG Wrightson Seeds, Ministry for Primary Industries, Rural Support Trust, New Zealand Veterinary Association and local veterinarians.

11 References

1. Collett, M.G., B. L. Stegelmeier, and B. A. Tapper. 2014 *Could nitrile derivatives of turnip (Brassica rapa) glucosinolates be hepato- or cholangiotoxic in cattle?* J Agric. Food Chem., 2014. **62**: p. 7370-7375.
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