VG126
Improvement of yield potential in
commercial fennel crops through improved
fruit set

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HAL

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Summary and recommendation:
Exogenous hormone trials suggested that intra-specific competition within the fennel canopy is limiting fruit set. Three factors were examined in order to assess their influences upon competition within the canopy.

Light is often one of the most limiting variables to the growth of a crop. This study showed that light intensity post initiation does not affect the number of umbels produced by a fennel plant. This does not rule out pre-initiation shading as a determinant of umbel number. The vegetative base may be an important influence on reproductive development.

Fennel does not show physiological adaptations to shading except under conditions of extreme shading. It originated in the Mediterranean region where clear skies and high light intensities are normal. Consequently it has evolved without selective pressures favouring adaptation to shade. The canopy is very open allowing good light penetration to all the umbels. Umbels of fennel have been found to be virtually independent of the vegetative canopy as far as photosynthesis and photosynthate is concerned. Therefore although competition for light probably exists within the canopy it is not likely to be limiting to growth. Light penetration is high enough that the vegetative canopy receives relatively high levels. This may be due in part to the fact that the leaves senesce as reproductive development continues, allowing even greater penetration of light to ground level. The open canopy is a morphological adaptation to avoid self-shading. Fennel would be a poor competitor for light with other more vigorous plants.

Fennel is quite drought tolerant. Its deep root system allows it access to water deep in the soil profile. Glasshouse fennel was able to withstand soil water potentials of less than -15 bars. Plants subjected to these soil water tensions showed leaf abscission but the flowers remained functional. It was not possible to show whether or not osmotic adjustment was a factor in enabling the plants to withstand such high water stresses.

The pollination ecology of fennel is probably the most important factor involved in the poor fruit set of fennel in Tasmanian commercial fields. Commercial crops tend to have very high planting densities which restricts the production of higher order umbels and in turn reduces the overlap between pollen production and stigma receptivity. It is hypothesised that reducing the planting density will result in higher percentage fruit set but this must take into account economic returns per unit area. It may be that incomplete fruit set on many plants produces a better return than
complete fruit set on fewer plants. An alternative to increasing plant density may be to plant fennel in beds separated by uncropped pathways. Plants on the edges of these beds will produce more of the higher order umbels which can then act as pollinators for the remainder of the crop. This should also produce a more uniform crop since the bulk of the crop will only be producing primaries and secondaries.

What is Fennel?
There is some debate over the taxonomy of the species *Foeniculum vulgare* Mill., commonly known as fennel. A bitter fennel variety was the subject of this work. Bitter fennel produces an essential oil with a characteristically bitter taste.

Fennel is a member of the family Umbelliferae which is synonymously known as the family Apiaceae. All members of this family are characterised by having their flowers arranged in umbels. Other members of the family Umbelliferae include the carrot (*Daucus carota* L.), dill (*Anethum graveolens* L.), parsley (*Petroselinum* spp.), coriander (*Coriandrum sativum* L.) and celery (*Apium graveolens* L.)

Why is Fennel Grown?
Bitter fennel is grown commercially for its essential oil which is a source of anethole, \((\text{C}_{10}\text{H}_{12}\text{O})\). Anethole is an aniseed compound used in the flavouring industry. The oil is obtained by steam distillation of the fruits and is approximately 60-70% anethole by weight. Although it is produced in all parts of the plant the bulk of the oil is produced in the fruits.

The fruits of fennel are schizocarps which are hard, dry and indehiscent. At first glance they appear to be bare seeds but they are by definition fruit since they are formed from the mature ovary wall.

What was the original problem giving rise to the research?
Commercial crops in Tasmania were found to be setting less than their total fruit potential. Since the fruit is the commercially valuable part of the crop, returns to the grower could be improved by increasing the fruit yield. The underlying theme of this work was to examine the agricultural ecology and physiology of fennel with the intention of finding means to improve the yield of fruit from fennel crops in Tasmania.

Background work by other authors?
Originally the general feeling by growers and others in the field was that there was a problem with pollination. This was thought to be due to competition from weed
species which were flowering at the same time as the fennel. It was thought that the bees were pollinating the weed species in preference to the fennel. The theory was discounted following work by Giudici (Honours thesis, Uni of Tas.) who found that if bees were absent from a crop, other insects, particularly flies were present to act as pollinators. Fennel is a promiscuous plant ie it does not have a specialised pollinator but has many features which make it compatible to pollination from both insects and wind. Insects are attracted by its yellow colour and its abundant nectar and pollen. The flowers are raised above the vegetative canopy making wind a suitable pollinator.

**Work Undertaken.**

This work set out to look at some of the physiological problems which could be reducing fruit set.

Two large scale, exogenous hormone application trials were conducted, following up on work by Roberts and by Menary and Hofman.

Unpublished work by N. Roberts, using radioimmuno assay techniques showed that the wild fennel which is high fruit setting differs significantly in its endogenous hormone balance from the lower fruit setting French varieties grown commercially in Tasmania. Three major differences were detected between the wild and cultivated varieties:

1) Prior to anthesis the endogenous auxin levels of the wild strains were significantly higher than those of the French varieties. From anthesis onwards the wild variety showed a slight increase in auxin while the French variety showed a dramatic increase finally reaching the levels of the wild fennel at the post anthesis stage.

2) There was a significant increase in the GA levels associated with wild fennel at anthesis, followed by a significant fall post anthesis. This trend was absent in the French variety; its GA levels remained constant throughout.

3) The ABA levels of the wild fennel increased from pre-anthesis through anthesis to post anthesis. The increase in ABA in the French variety was much lower and reached its maximum at anthesis with no further rise.

A field trial by R. Menary (unpublished) involved spraying fennel with various concentrations of auxin at various stages of flower development. The experiment produced no significant results, the variation within the blocks being great enough to disguise any possible effects. There were however trends in the data which suggested that fruit set in fennel may be manipulated by application of exogenous
auxin prior to anthesis. 200ppm β-naphthoxyacetic acid proved toxic to fennel but 50ppm and 100ppm had the effect of increasing the number of fruits present. There was no increase in the yield of fruit by weight because the average fruit weight was reduced.

Results of the Current Trials.
In both trials the hormones were applied to plants growing as part of a commercial crop. The treatments were applied when the average stage of development of the crop was at anthesis of the secondaries.

The first trial was conducted at the University Farm, Cambridge.

key to treatments:
1. 100ppm β - naphthoxyacetic acid (β - NOA)
2. 100ppm p - chlorophenoxyacetic acid (p - CPA)
3. 100ppm α - naphthoxyacetic acid (α - NOA)
4. 50ppm GA₃ + 100ppm β - NOA
5. 100ppm GA₃ + 100ppm β - NOA
6. control

The results are displayed in figure 1. The auxin treatments had no significant effect on the weight of seed per plot. The GA₃ treatments produced two effects which were significant at the 5% level. The first was to reduce the weight of fruit obtained from the primaries and the
second was that the 100 ppm GA$_3$ treatment increased the weight of fruit per plot from the tertiaries. The GA$_3$ induced a change in the competitiveness of the sinks, reducing the dominance of the primaries and increasing the strength of the tertiaries.

The second trial was conducted on a small experimental plot at Glenleith, Plenty. The treatments applied in this trial are as follows:

1. 100 ppm $\beta$ - NOA + 25 ppm GA$_3$
2. 100 ppm $\beta$ - NOA + 50 ppm GA$_3$
3. 100 ppm $\beta$ - NOA + 100 ppm GA$_3$
4. control

Several measurements were made and the results are displayed in figures 2 - 6.

The number of leaves per stem were counted at a preliminary harvest, before the crop reached full maturity. It was found that the GA$_3$ treatments increased the longevity of the leaves by delaying their senescence. All treatments had significantly higher numbers of leaves than the control, at the 5% significance level. There was no significant difference between the treatments. The number of leaves at this early harvest are displayed in figure 2. By the time of the final harvest all the plants had shed their leaves.

The number of umbels per plant was assumed to be constant throughout the experiment since the treatments were applied after the buds had emerged from their sheaths. Any effect on yield was assumed to come from an increase in fruit set and
not from an increase in umbel initiation or development.

The total yield of fruits per plot shows a trend towards being reduced by the applied treatments. Refer to figure 3. The result is significant at the 10% level. The treatments significantly increased the average fruit weight however the effect was decreased by increasing levels of GA$_3$. Refer to figure 4. At the 5% significance level the control was significantly lower than both the 25 and 50 ppm GA$_3$ treatments and the 25 ppm GA$_3$ treatment was significantly higher than the 100 ppm GA$_3$ treatment.

It is surmised from this that the overall fruit yield is reduced due to a reduction in the relative weight of fruit from the primaries but that the average fruit weight is
increased because the weight of individual tertiary fruit is increased. The number of tertiary fruits per plant is greater than the number of secondaries which is in turn greater than the number of primaries ie the number of fruits per umbel order reflects the number of umbels per plant of each umbel order.

Despite a very distinct trend in the data indicating that the applied treatments decreased the yield of oil from the plots, (figure 5), the analysis of variance showed the treatments to have had no significant effect. Least significant means analysis showed the yield of oil from the control to be marginally (at the 10% level) higher oil yields than the plots treated with hormone.

The anethole content of the oil was not significantly affected by the applied
treatments, even at the 10% level. Least significant means analysis showed no differences between any of the treatments. The trend which appears in the graph (figure 6.) is not significant.

Increasing the number of modules in the canopy ie the number of leaves and fruits, appears to have decreased the overall yield in terms of both weight of fruit and oil. Much of the variability in the experiment was due to plants missing from the plots. Unfortunately this was not apparent until after the harvest. Fennel produces compensatory growth in order to fill as much of the empty space as possible, and the experimental area appeared to be uniform until the canopy was removed at harvest. It then became obvious that in some plots many of the stems were arising from common root systems. Consequently it is believed that statistically the trends in the data may be masked by the variability and that acceptance of a higher than usual probability level is justified.

The acceptance of the hypothesis that increasing the number of modules within the canopy decreased the yield from the plots lead to an hypothesis of intraspecific competition being the cause for less than full potential yield from fennel in dense situations eg those experienced in commercial crops in Tasmania.

Any growth factor may be the subject of intraspecific competition but two were chosen as being likely to have greater influence on fennel yields. These two were light and water. In a further experiment, a trial was conducted to study the effect of density on the relationship between the time of pollen production and the time of stigma receptivity. The results from this gave rise to the further hypothesis that intraspecific competition for pollen exists.

![figure 7. The effect of increasing levels of shading on the production of umbels.](image-url)
Light intensity and its effect on flowering in fennel.

In a glasshouse trial, fennel plants were shaded during umbel development to assess the effect on umbel number due to shading at high planting densities. Plants were placed under varying shading regimes post flower initiation. The results are presented in figure 7.

Statistical analysis of the data showed that, post initiation, light intensity does not affect the number of flowering umbels unless it is so low that the plant is prevented from growing.

The effect of shading on the photosynthetic capacity of fennel was examined by generating light response curves for plants grown under shade cloth conditions similar to those in the above experiment. The entire light response curves are
presented in figure 8. In order for the light compensation points to be more clearly observed the linear portions of these curves are then displayed in figure 9.

Dark respiration is lower in plants grown at very low light levels but moderate shade has no effect. The light compensation point is also lower for severely light stressed fennel, but it is unaffected by moderate shading.

The transmission of light through the flowering canopy of fennel in the field was examined in order to compare it with the above glasshouse and laboratory data. See figure 10 for the results.

For all the planting densities measured the primaries were receiving approximately 80% or greater of the incident light. This is higher than the light received by all the shading treatments in the previous experiment. The whole flowering canopy intercepts at most approximately 50% of the light which is equivalent to the lowest shading treatment applied. ie The fennel flowering canopy is very open, allowing good penetration of light and the umbels should not be affected by low light levels even in dense stands.

The vegetative canopy absorbs light to allow 20 - 30% to reach ground level. At 4 plants/m² the value is higher due to incomplete canopy closure. Levels of 20 - 30%
are equivalent to or slightly lower than the medium shading treatment in the above experiments. Although competition for light exists, at the densities tested it is unlikely that light intensity was low enough to significantly affect flower number in the post initiation phase.

**Water Stress.**

Fennel is an apparently mesophilic plant with its origins in the Mediterranean Basin. The climate in this region is characterised by cool, wet winters but hot and dry summers. It is normal for plants growing in this area to display at least some xerophytic characteristics, consequently it is believed that fennel is a phreatophyte.

Phreatophytes are an ecological group of plants which grow using the water of the water table and/or the capillary fringe found just above it. They are a mesophilic group of plants which grow in semi-arid or arid environments. Their most distinctive characteristic is their deep root system which is necessary for them to reach the underground water. They often have very high water usages since they can tap a plentiful source of water. Other characteristics which allow such plants to cope with above ground water stress include both diurnal and seasonal osmotic adjustment. They have the ability to withstand water stress while allowing their stomata to remain open and photosynthesis to continue.

Fennel is known to have a long tap root and very large root system. It was found by Clark (unpublished), using a neutron probe, that commercially grown fennel at Glenleith was drawing water from a depth of 1.2 m. A neutron probe was used to assess the water use by a density trial planted at the University Farm. The soil in which this trial was planted was strongly duplex with a sandy upper layer and heavy clay below. Water utilisation was not as deep as expected, probably due to the duplex nature of the soil.

Plants growing at a planting density of 4 plants/m² were found to obtain most of their water from the first 60 cm of the soil profile. There was some water usage to a depth of 100 cm. Plants at 100 plants/m² obtained most of their water requirements in the first 40 cm of the profile and although there was some usage above this it was much less than for the 4 plants/m². Two plots of 25 plants/m² were measured with one tending to the 4 plants/m² and one to the 100 plants/m². The overall water usage was highest for the 4 plants/m² which is thought to be due the higher number of leaves on these plants.

In order to gain a better understanding of the effect of water stress in fennel a
A glasshouse trial was designed which would subject plants to water stress under controlled conditions.

Applying water stress to plants in a controlled manner is difficult. Two general ways in which this can be done are either to withhold water or to increase the soil water potential by using a high molecular weight osmotic medium e.g. polyethylene glycol. This second method is often used but has two significant drawbacks. The first being that some lower molecular weight compounds may be absorbed by the plant and prove toxic. The water stress effects can be confounded by the toxicity. The second drawback also relates to the toxicity. Water stress cannot be applied quickly to a plant but must be increased slowly in order that the plant is not damaged by osmotic shock due to the treatments rather than the water stress.

The best, most natural, method for applying water stress to plants is to withhold water for a certain period of time or until the plant receives a certain degree of stress. Since environmental conditions are variable and they effect the degree of water use by a plant, withdrawing water for a fixed period causes varying degrees of stress. A better method is to withhold water until a fixed level of stress is reached. This stress may be measured through objective means such as leaf water potential, relative water content or soil moisture content.

For this experiment it was decided that water be withheld until the soil water potential had reached a certain level and then to rewater the plants to field capacity. Measurement of soil moisture could have been made with a tensiometer but this would only measure a specific region in the pot and also offered only a limited range of water potentials.

It was decided to use the percentage soil moisture by weight to control the stress received by the plant. In order to do this plants were grown in a known volume of soil until they flowered and the experiment ready to commence. They were watered and then allowed to drain, in the dark, overnight before being quickly weighed to obtain the weight of the pot, plus soil, plus plant, at field capacity of the soil. From this measurement, a knowledge of the amount of soil in the pot and the water capacity of the soil it was determined how much weight the pot must lose in order for the soil to reach a certain percentage water content. Water potential curves had already been produced for the soil which showed the water potential of the soil at a given water content.

A weighing system was constructed to continually weigh the plants. The balance
was set to tip when the potted plant had decreased in weight to the level desired. At this point the plant was re-watered to field capacity.

The system was found to be reliable with the balances having a sensitivity of ±20g. Since the required weight loss was in the order of 500g for the least stressed treatments and 1.5kg at the other end of the scale this error was quite acceptable.

A disadvantage of the system was the fact that the balances had to be recalibrated regularly due to the fact that the plants were growing and therefore putting on weight. At the flowering stage, which is the stage at which these plants were studied, they accumulated approximately 10 - 20g fresh weight per week. This error was considered acceptable however the method could not have been used during the phase of rapid elongation since the increase in plant weight would have been too high.

One disadvantage which was not taken into account in this experiment is the loss of weight due to loss of water from the plant. No measure of this was made when conducting the experiment.

The plants were arranged into three treatments, with the following re-watering points;

a) low water stress, soil moisture 22%, soil water potential -6atm.
b) medium water stress, soil moisture 20%, soil water potential -10atm.
c) high water stress, soil moisture 10%, soil water potential <-15atm.

Once the plants had been acclimatised to these soil moisture contents, leaf samples were taken just prior to re-watering (ie at peak water stress) in order to assess the relative water content of the plants at this level of stress. It was found that the average relative water content for plants in each treatment was 95%, 93% and 86% for low, medium and high stressed plants respectively.

Osmotic adjustment allows plants to adapt to water stress. It was hypothesised that increasing water stress would cause increasing osmotic potential in the leaves. It is usual for this to be detected by using a pressure bomb to produce a pressure/volume curve for the leaf. The y-
intercept of the linear portion of the curve gives the osmotic potential of the leaf. A typical curve has the shape illustrated in figure 11, which also shows how the osmotic potential (\(\pi\)) is derived from the water potential (\(\Psi\)) (equivalent to the pressure applied by the pressure bomb) and the volume of the sap droplet expressed (\(v\)).

For a plant with very soft tissue, such as fennel, the pressure bomb tends to crush the stem giving inaccurate results. For this reason a relatively new method was used to produce the pressure volume curves. Leaf segments were equilibrated with salt solutions of various water potentials and the relative water content of each equilibrated segment was plotted against the reciprocal of the water potential of the salt solution to which it was equilibrated. This curve is the same shape as the one in figure 11 and the osmotic potential can be determined in the same manner.

Unfortunately this technique also has inherent problems. The most common being that condensation forms on the surface of the leaf discs which affects both weight measurements and leaf equilibration. It did not appear to be a problem in this experiment however the results were extremely variable, even after several replications. The problem could also have been due to the very small amounts of tissue being weighed. Small errors in weighing would be relatively large when compared with the weight of material being used. This was unavoidable since to obtain large amounts of tissue would require the use of more than one leaf which in turn introduces variability due to the non-uniformity of the tissue.

No differences could be detected in osmotic potentials of the fennel leaves from the water stress treatments and at this stage it is not possible to tell whether or not osmotic adjustment is absent or whether it is present but not detectable by this method.

Pressure bomb curves were drawn up later in the season but they are not considered reliable because the tissue was crushed by the pressure bomb and also because the plants were old when the samples were taken.

Pollen production, stigma receptivity and planting density.
Fennel is protandrous, meaning that on each individual flower the anthers dehisce, releasing their pollen, before the stigma becomes receptive to pollination. This is a mechanism to prevent self-pollination. However the outside flowers of the umbel mature before the inside flowers. Pollen production progresses in a wave from from the outside inwards, followed by a wave of stigma receptivity. These waves overlap...
so that the inside flowers are producing pollen while the outside flowers have receptive stigmas. Therefore some self-pollination does occur. Self-pollination is also enhanced by the fact that the maturity of the umbel orders is also in waves. The primary umbel matures first, followed by the secondary and then the tertiary etc. Pollen is transferred from the outer flowers of the secondary to the inner flowers of the primaries etc.

An experiment was designed to investigate the relationship between pollen production and stigma receptivity, especially across the umbel orders. Since density affects the number of umbels, it was hypothesised that at high planting densities, where umbel number per plant is low, there would be less overlap between pollen production and stigma receptivity than at low planting densities where the umbel number per plant is high.

The results of this trial are graphed in figures 12 - 15. These histograms show two columns for each sampling date. The first column shows the number of umbels (per m\(^2\)) with anthers which are releasing pollen. The second column shows the number of umbels with receptive stigmas.

Fennel pollen is viable for only a few hours following anther doziness. In order for pollination and fruit set to be successful, the pollen must be transferred to the stigma immediately. This means that for fruit set to occur pollen must be available simultaneously with stigma receptivity. All the graphs (figures 12 - 15) show a balance between pollen production and stigma receptivity early in the season. The graphs for planting densities of 4 and 12 plants/m\(^2\) (figures 12 and 13) show that at these low planting densities, where the number of tertiary umbels is high, there is adequate overlap between pollen production and stigma receptivity to produce good fruit set, even in these higher order umbels. The graphs for the 50 and 100 plants/m\(^2\) plots (figures 14 and 15) show adequate overlap for pollination of the primaries and early maturing secondaries but later in the season an imbalance arises as the umbels move from pollen production to stigma receptivity. There are inadequate umbels of the higher orders eg teritories to become pollen producers and competition for pollen is strong.

A Possible Bacterial Disease in Fennel:
Throughout this work there was a recurring problem with what appeared to be a bacterial disease attacking the plants.
figure 12. Histogram showing the number of umbels/m², of each umbel order having flowers either at anthesis (producing pollen) or with receptive flowers either at anthesis (producing pollen) or with receptive stigmas, at each sampling date, for a planting density of 4 plants/m².

figure 13. Histogram showing the number of umbels/m², of each umbel order having flowers either at anthesis (producing pollen) or with receptive flowers either at anthesis (producing pollen) or with receptive stigmas, at each sampling date, for a planting density of 12 plants/m².
figure 14. Histogram showing the number of umbels/m², of each umbel order having flowers either at anthesis (producing pollen) or with receptive flowers either at anthesis (producing pollen) or with receptive stigmas, at each sampling date, for a planting density of 50 plants/m².

figure 15. Histogram showing the number of umbels/m², of each umbel order having flowers either at anthesis (producing pollen) or with receptive flowers either at anthesis (producing pollen) or with receptive stigmas, at each sampling date, for a planting density of 100 plants/m².
In the young vegetative plant, the symptoms appeared most frequently, under overhead watering. In these plants the most common symptom was the rotting of the young, emerging leaf.

In older plants the leaves of diseased plants showed necrotic tips with a water soaked region at the boundary of the necrotic area and the healthy tissue. Other symptoms included thickening of regions within the stem to give it a bumpy texture. Stem lesions also occurred infrequently.

The most destructive symptom was the abortion of the flower buds. These symptoms appeared very similar to myrad attack but with no sign of myrads present and no sign of probing holes where they had been feeding.

In the glasshouse copper spray and hand or dripper watering helped to control the symptoms on the young plants but no success was achieved in controlling the attack on the older, flowering plants.

Plants which were under stress seemed to be more susceptible but not all stressed plants succumbed to the disease. The diseased plants occurred randomly in the glasshouse and were never concentrated on one bench or in one area; healthy and diseased plants occurred side by side.

The disease was not related to aphid or two-spotted mite attack, the two most common pests in the glasshouse. The symptoms occurred independently of these, with some plants heavily infested with pest succumbing and vice versa. Similar symptoms were observed on plants in the field, however in the field diseased plants tended to occur in patches.

Using a microscope, bacteria were observed streaming from the cut edge of a necrotic leaf. Gram negative, motile rods were isolated from similar samples.

Several colony types were isolated from both glasshouse plants and field samples. In particular, one unusual bright yellow colony type was isolated from several different field samples. The samples were taken aseptically, but they were taken on the same day so external contamination cannot be completely ruled out. This colony type was not isolated from any glasshouse plants.

None of the colony types isolated produced lesions when reinoculated into leaves of healthy plants in the glasshouse. This does not necessarily mean that none were
pathogens as specific environmental conditions may be necessary to cause the initial inoculum to develop into the disease. It may also be that the pathogen did not withstand the isolation procedure.

At this stage nothing conclusive can be said about the disease observed. It may or may not be a bacterial disease but given that in commercial fields the plants showing the symptoms can be abundant, future work should be undertaken to sort out this problem.

Extension and Adoption by Industry:
During the course of this investigation field officers of Essential Oils of Tasmania (E.O.T.) have been involved in observing the results of trials and their possible application to commercial production. To date the principles have been accepted but not translated to field demonstrations.

Direction of Future Research:
The work on row thinning to produce a pollen source for the higher order umbels should be a future activity for HRDC and EOT.

In addition, trials should be conducted with copper compounds to determine the effect of bacterial infection on fruit set.

Financial or Commercial Benefits:
Trial results indicate that yield increases between 50 and 100% can be attributed to controlling the lack of pollination and bacterial infection of flowers.