Modelling cell separation during plant organ abscission

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Abstract: A simple mathematical model is presented to describe the cell separation process that plants undertake in order to deliberately shed organs. The focus here is on modelling the production of the enzyme polygalacturonase, which breaks down pectin that provides natural cell-to-cell adhesion in the localised abscission zone. A coupled system of three ordinary differential equations is given for a single cell, and then extended to hold for a layer of cells in the abscission zone. Simple observations are made based on the results of this preliminary model and, furthermore, a number of opportunities for applied mathematicians to make contributions in this subject area are discussed.

1 Introduction

Abscission is the process in which plants intentionally shed their leaves, flowers, buds, seeds, fruit, branches or roots. This may occur for a variety of reasons, for example if the organ is senescing or stressed. If a leaf is attacked by a pathogen, or becomes diseased, a plant may quickly shed the organ rather than going through the usual (longer) process of recycling nutrients. Seeds may be abscissed to continue plant propagation, or if infested by insects. Flowers may be shed after pollination, perhaps to encourage insects to visit unpollinated flowers. In a deciduous plant, the leaves are shed during autumn/winter to help survive the cold climate, or before the dry season, in order to conserve water.

A motivation for studying the mechanical and biochemical components of abscission comes from the desire to manipulate the process, with benefits for the agricultural and horticultural industries. For example, there may be some advantage in accelerating the abscission of ripe fruit (for successful harvesting) and young fruit (at the end of a season, to protect the plant from pathogen attack). Alternatively, one may wish to delay (or prevent) the abscission of healthy leaves.

The abscission process involves the breakdown of the cell-cell adhesion in a layer of cells known as the abscission zone. In this report we present a simple mathematical model for the production and secretion of enzymes that are responsible for this cell wall degradation, and discuss further avenues for research in this area.

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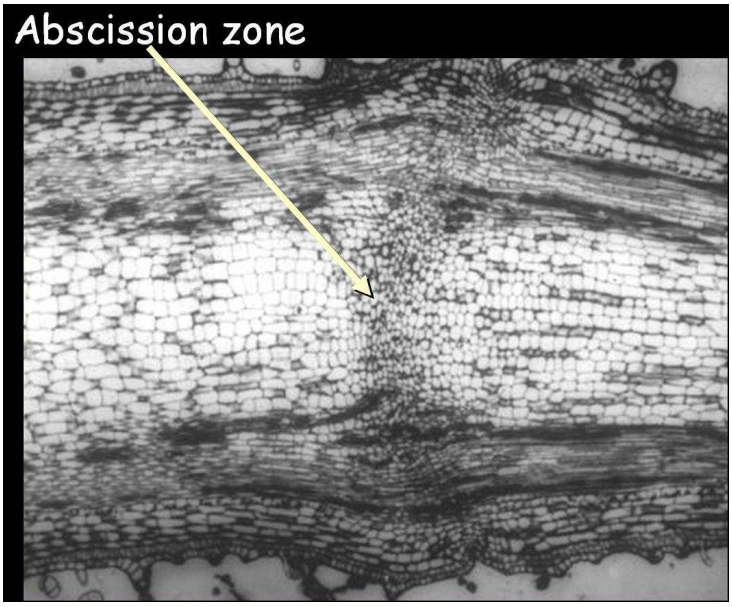

Figure 1: Light micrograph showing abscission zone in tomato flower. In this figure the proximal tissue is to the left, and the distal to the right. Taken from [5]

2 Key biological processes

In this section we provide a brief overview of the most important morphological properties and biochemical processes involved in organ shedding. We shall not give an extensive survey with specific citations, but instead provide a brief summary, emphasising the important points from a modelling perspective. For detailed references on the following mechanisms, the reader is referred to the monographs [1, 14] or the review articles [5, 7, 9, 10, 12, 13], for example.

2.1 Properties of abscission zone

Abscission occurs by cells separating in a predetermined cellular layer, known as the abscission zone, normally found at the base of the organ that is to be shed. An example of such as zone is shown in Figure 1, which is taken from a tomato plant [5]. As can be seen in this micrograph, the cells in the abscission zone are smaller in size, and grouped together in a layer that is slightly domed in shape. Furthermore, these cells are more isodiametric, and less elongated than those adjacent to the abscission zone. Such properties hold more generally in other plant species, although the width of the abscission zone may vary between roughly five and 30 cells.

There is evidence to suggest that cells in the abscission zone have certain other defining characteristics (as opposed to cells either side of the abscission zone), such as:

- they are more densely cytoplasmic;
- they have smaller intercellular spaces, with possible differences in the make up of the cell walls;
- they respond differently to plant hormones such as ethylene and auxins (see Subsection 2.3);

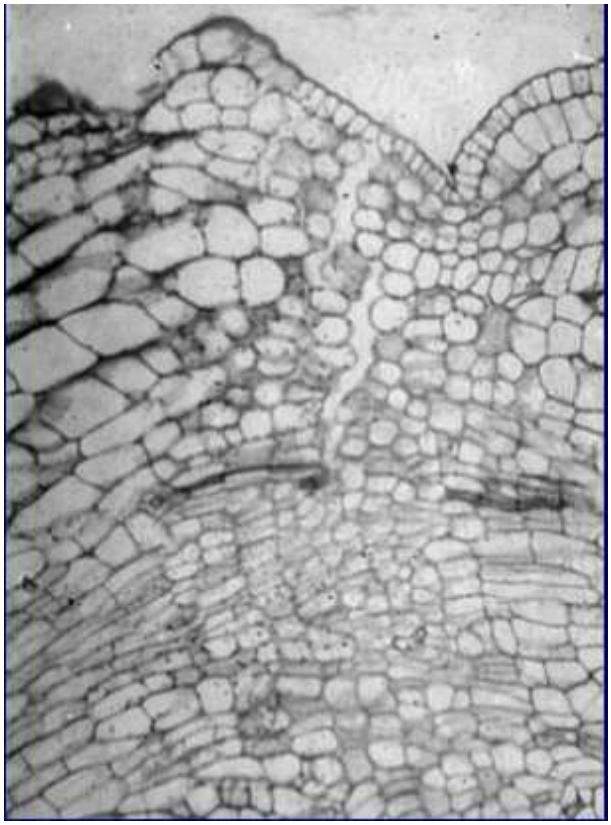

Figure 2: Light micrograph showing abscission in tomato flower. In this figure the proximal tissue is to the left, and the distal to the right. Taken from [5].

- they have enhanced transcription of cell wall degrading enzymes such as polygalacturonases (see Subsection 2.4).

Some of these concepts will be expanded upon below.

2.2 Precise nature of abscission

A remarkable observation is that the distal part of the plant breaks off very cleanly, in a way that can be thought of as a "fracture". This shedding occurs without damaging cells in the neighbourhood of the fracture (we shall call this thin layer the *separation layer*). Indeed, even cells that are two or three rows away from the fracture line do not appear to undergo any significant cell separation. This phenomenon is illustrated in Figure 2, which shows a flower abscissing, and in Figure 3, which shows an imagine of tomato leaf abscission, taken with a scanning electron microscope (SEM). There is a question of whether the cells immediately each side of the separation layer are different in cell wall composition to other cells in the abscission zone, or whether the clean break can be driven by the differentiation of specific target cells for ethylene and indole-acetic acid (IAA), the latter being an auxin.

2.3 Hormone activity that triggers abscission

The timing of abscission is believed to be controlled by the concentration of two key plant hormones, ethylene and IAA. Ethylene, a gas that is synthesized by cells in the plant, is known

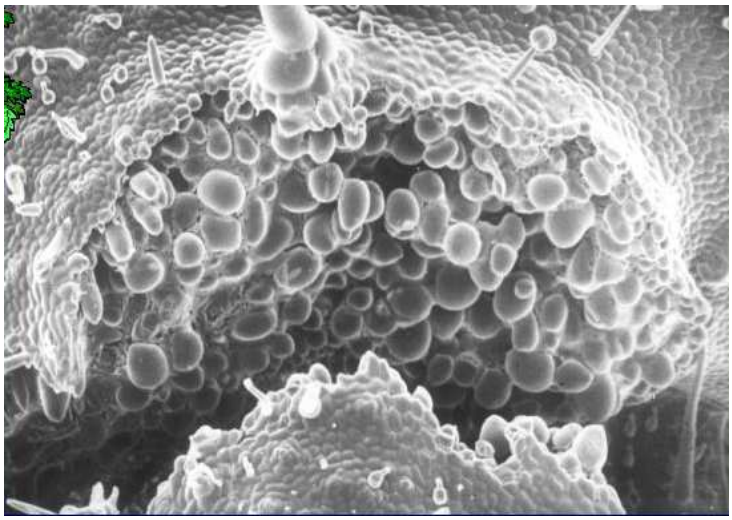

Figure 3: SEM image of tomato leaf abscission, taken from [8].

to promote organ shedding, with experiments showing that an increase in ethylene levels in the abscission zone can lead very quickly to abscission (the abscission that is pictured in Figure 2 is triggered by exposing the plant to ethylene). On the other hand, IAA acts as an inhibitor of abscission, with increasing levels shown to delay the process. It is the balance between the concentration of ethylene and the IAA flux through the abscission zone cells that is critical.

2.4 Enzyme activity leading to cell separation

Most plant cells remain attached to a particular array of cells throughout their life cycle. The cell walls contain the polymer pectin, a gelling agent which provides the cell-to-cell adhesion that keeps the plant cells connected. During abscission, the actual cell separation is brought about by the production of hydrolytic enzymes that break down the pectin (in the middle lamella, the layer between adjacent cell walls). For the purposes of the present study, the most important of these is assumed to be (certain isoforms of) polygalacturonase (PG), although cell wall loosening is also believed to be caused by the action of β-1,4-glucanase (cellulase) and expansins.

Experiments have shown an increase in PG activity in the abscission zone, although it is unclear whether it is only the cells in the immediate vicinity of the fracture that produce the enzyme, or whether it is produced in the entire zone. Indeed, this is a question we hope to address in our model.

A review of the role of PG in cell separation has been published recently [11].

2.5 After shedding

In addition to undergoing cell separation, the cells that comprise the abscission zone also exhibit other modifications. Many of these are related to protecting the fracture surface from invasion by opportunistic pathogens. These events include the synthesis of new cell wall material and pathogenesis resistance proteins [2]. These processes will not be considered in this document.

3 Simple mathematical model

3.1 Finding a tractable problem

We have chosen to focus on the abscission process after induction. We have assumed that this is temporally regulated by a balance between ethylene and IAA, with ethylene acting as a promoter, and IAA as an inhibitor. There is no evidence of a feedback from the cell to the production of either ethylene or IAA. Thus, for the purposes of developing our mathematical model, we have assumed that the entire process is switched on when the balance between ethylene and IAA reaches some critical level. We are not concerned with the details of this signalling process. We simply suppose that at $t = 0$ the cells in the abscission zone detect a signal that stimulates the production of PG, thus beginning the series of events that lead to organ shedding. As a consequence, we shall not consider either hormone (ethylene or IAA) in our model, but instead focus on the interaction between PG and the pectin in the cell wall. Matters relating to ethylene and IAA will be revisited briefly in Section 4.

We also assume that, in spite of their similarity, the cells in the abscission zone are not actually identical. Instead, we assume that during the development of the plant every cell in the abscission zone was pre-programmed to respond to the hormonal trigger in a specific manner such that the abscission process will proceed in a controlled way. The problem we are left to deal with concerns the precise manner in which cells must behave in order to achieve this controlled abscission.

Two general secretion patterns are conceivable: The cells in the abscission zone can either secrete PG uniformly in all directions or only towards the separation layer. To decide which of these patterns a plant actually uses is one of the major open questions that our modelling project seeks to address. The specific model described below assumes a uniform secretion in all directions. However, it will be obvious that the model can easily be adapted to investigate the consequences of directional secretion.

Our hypothesis is that, after receiving the hormone trigger, each cell in the abscission zone does indeed produce PG and subsequently secretes the enzyme into the cell wall. However, since the plant needs to control this process, we assume that each cell can detect the amount of pectic debris in its cell wall, and uses that information to regulate the flux of PG through the cell membrane. Furthermore, we hypothesize that the rate of PG production in a cell, and the flux of PG through the membrane, can vary from cell to cell in the abscission zone. In extreme cases, it could be zero, i.e., not all cells must actually secrete. The question then is what sort of variation in these properties is required in order for the abscission or fracture to occur at a discrete site, as observed in experiments.

3.2 Single cell model

For the moment consider a single cell in the abscission zone. We denote the concentration of PG in the cell by $P(t)$ and the concentration of PG in the cell wall by $Q(t)$, both of these quantities being dimensional. Further, we denote the proportion of pectin that is degraded by $R(t)$, noting that this nondimensional measure takes values in the range $0 \leq R(t) \leq 1$. The governing equations of our model are:

$$\frac{\mathrm{d}P}{\mathrm{d}t} = k - \alpha(R)P - \mu P, \tag{1}$$

$$\frac{\mathrm{d}Q}{\mathrm{d}t} = \alpha(R)P - \gamma Q, \tag{2}$$

$$\frac{\mathrm{d}R}{\mathrm{d}t} = \beta Q\,(1 - R)\,. \tag{3}$$

We assume that PG is produced by the cell at a constant rate k. In (1) and (2), the term $\alpha(R)P$ is the rate at which the cell secretes the PG into the cell wall. The cell regulates the flux of PG into the cell wall and halts the process once the critical amount of pectin has been degraded. This feedback is modelled by the function $\alpha(R)$, which is a decreasing function with $\alpha(0) = \alpha^*$ and $\alpha(R^*) = 0$, where R^* is the critical amount of degraded pectin. We shall suppose that

$$\alpha = \begin{cases} \alpha^* \left(1 - \dfrac{R}{R^*}\right)^m & \text{for } R < R^*, \\ 0 & \text{for } R > R^*, \end{cases} \tag{4}$$

where the exponent $m > 1$ is included to ensure that the rate at which α decreases with respect to R is smooth for all R. The terms μP and γQ are included in (1)-(2) to model the cell "mopping up" excess PG before it degrades other components of the plant, the mechanism for which could be regulated by a polygalacturonase inhibitor protein. Typically we would expect $\gamma > \mu$, although below we simply set each parameter to be unity. Finally, the rate at which the pectin is degraded, $Q\,(1 - R)$, is linearly dependent on Q, the amount of PG in the cell wall, and $1 - R$, the proportion of pectin that is intact.

A quick check of (1)-(3) shows that a steady state solution is given by $P = k/\mu$, $Q = 0$ and $R = R^*$. To run a numerical simulation, we use the initial conditions $P(0) = 0$, $Q(0) = 0$ and $R(0) = 0$, with $t = 0$ representing the time at which the plant begins producing PG in response to the hormone signal. A typical plot of the behaviour of the dependent variables is given in Figure 4. We see that R increases monotonically from zero to the steady state value R^* that, by hypothesis, the cell seeks to achieve. The secretion rate first increases and then decreases as the degradation rate approaches its limiting value and the cell reduces the secretion of PG. The concentration P of intracellular PG approaches the steady state limit k/μ for long times. As no more PG is secreted, an actual cell would presumably also reduce the production of PG and reduce the intracellular concentration back to zero. A mechanism to achieve this reduction is not included in our model. It is not required because we are mainly interested in the degradation of the cell wall, which is the observable quantity. It would be straight forward to include a more accurate model of the intracellular processes, but this would not contribute to the important features of our model.

It is important to stress that Figure 4 is representative only, showing the key qualitative features of the model (1)-(3). As such, the parameter values chosen for these plots were not taken from any experimental study, and the actual dimensional values taken by P, Q and t are not indicated. To calibrate the parameter values with measured quantities in a particular plant would require a substantial experimental component that is beyond the scope of this preliminary report. This is, of course, an important feature of the overall study, and we leave it for further research.

3.3 Multiple cell model

We can extend the above model by considering a one-dimensional array of cells running perpendicular to the abscission zone, as indicated in Figure 5. The ith cell will have a PG concentration $P_i(t)$, while the notation $Q_i(t)$ will be used to denote the concentration of PG in the cell wall between the cells $i - 1$ and i. Similarly, R_i denotes the proportion of pectin that is degraded in the wall between cells $i - 1$ and i. This notation is indicated schematically in Figure 5. Using this approach we can track the dependent variables across the abscission zone. We assume that the proximal direction corresponds to decreasing values of the index i, while the distal direction corresponds to increasing values. We shall also assume that the two middle layers of cells are preprogrammed so that the abscission will occur between their walls.

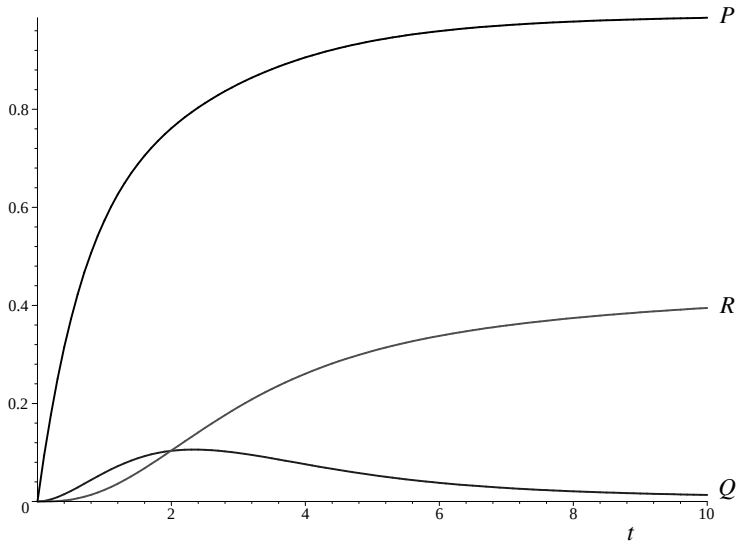

Figure 4: The dependence of P, Q and R on time t for the single cell model, calculated with $k = 1$, $\mu = 1$, $\alpha^* = 1/4$, $m = 2$, $R^* = 1/2$, $\gamma = 1$ and $\beta = 1$.

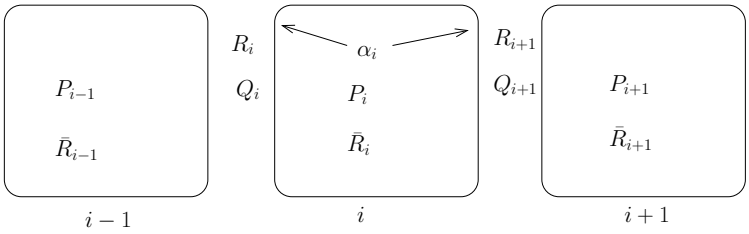

Figure 5: Labelling of quantities in one-dimensional array of cells.

To generalise our single cell model, we need to specify how a cell regulates its secretion of PG in response to the pectin degradation in both adjacent cell walls. Earlier, in the single cell model, we used the form (4), which described the dependence of α on R, the proportion of pectin degraded in the single cell wall. In a one-dimensional array of cells, this proportion for the ith cell should be taken as some sort of average of that quantity in the proximal and distal direction. As a first approximation, we therefore suppose that

$$\alpha_i = \begin{cases} \alpha^* \left(1 - \dfrac{\bar{R}_i}{R^*}\right)^m & \text{for } R < R^*, \\ 0 & \text{for } R > R^*, \end{cases}$$

where

$$\bar{R}_i = \frac{1}{2}(R_i + R_{i+1}).$$

Our multiple cell model becomes

$$\frac{\mathrm{d}P_i}{\mathrm{d}t} = k - \alpha_i P_i - \mu P_i, \tag{5}$$

$$\frac{\mathrm{d}Q_i}{\mathrm{d}t} = \frac{1}{2}(\alpha_{i-1}P_{i-1} + \alpha_i P_i) - \gamma Q_i, \tag{6}$$

$$\frac{\mathrm{d}R_i}{\mathrm{d}t} = \beta Q_i \left(1 - R_i\right), \tag{7}$$

with initial conditions $P_i(0) = 0$, $Q_i(0) = 0$, $R_i(0) = 0$. Note that since Q_i denotes the concentration of PG in the cell wall *between* cells $i-1$ and i, we have taken the rate at which the adjacent cells secrete the PG into this cell wall to be the average of the rates $\alpha_{i-1}P_{i-1}$ and $\alpha_i P_i$ (see equation (6) above).

A key question is which of our parameters will vary along the array of cells, and what form this dependence will take. In the first instance we may assume that k depends on i, but that the other parameters take the same value in each cell. For this case we write k_i as the constant rate of production of PG by the ith cell. A reasonable assumption could be that this production is greatest at the centre of the abscission zone, and decreases in both distal and proximal directions.

With this in mind, the coupled system (5)-(7) was solved numerically with the fixed parameter values $\mu = 1$, $\alpha^* = 1$, $m = 2$, $R^* = 1/2$, $\gamma = 2$ and $\beta = 1$, and the dependence of P_i, Q_i and R_i on time are shown in Figure 3.3. These plots were generated using 10 cells with k_i given by

$$k_i = \frac{1}{\sqrt{2\pi}} \mathrm{e}^{(i-N/2)^2/2} \tag{8}$$

for $i = 1..10$, which approximates a normal distribution. Noting that, for this preliminary example (for which we have forced all parameters except k_i to be constant across each cell), the problem is symmetric about the middle two cells. As such, only results for the first five cells are shown (although on the scale used, some of these plots are not visible). These results are only representative, as no effort has been made to estimate the parameters in question. But it is clear that if cells do produce PG at a rate which is maximum at the abscission zone and decays on either side (either through some pre-programming or through artificial intervention), and if all other parameter values remain fixed, then the predicted amount of pectin broken down is greatest in the middle of the zone.

This conclusion is not surprising, since we have deliberately forced the cells in the centre of the array to produce more PG, thus leading to a higher level of pectin degradation there. In reality, it may be unreasonable to assume that the processes are all symmetric about the fracture line. Indeed, it is presumably in the plant's interest to protect the tissue on the proximal side of the fracture, thus cells on the proximal side of the one-dimensional array may secrete PG into the surrounding cell wall at a lower rate than at the distal side. A way to facilitate this idea could be to suppose that the parameter R^*, the critical amount of degraded pectin below which no PG is secreted into the cell wall, is a monotonically increasing function of i. It would be interesting to solve the system (5)-(7) with this assumption, together with values of k_i such as (8); this task is left for future research.

4 Discussion and further research

4.1 The present study

We have presented a very simple mathematical model for the production of cell wall degrading enzymes involved in the abscission process. In this model, the abscission zone is represented by a one-dimensional array of cells, whose properties may vary in both the proximal and distal directions. The key feature of the model is that the rate at which a cell secretes polygalacturonase into the cell wall is controlled by the concentration of degraded pectin there. This feedback mechanism is built in to the single cell model (1)-(3) and the multiple cell model (5)-(7) through the function $\alpha(R)$, defined in (4).

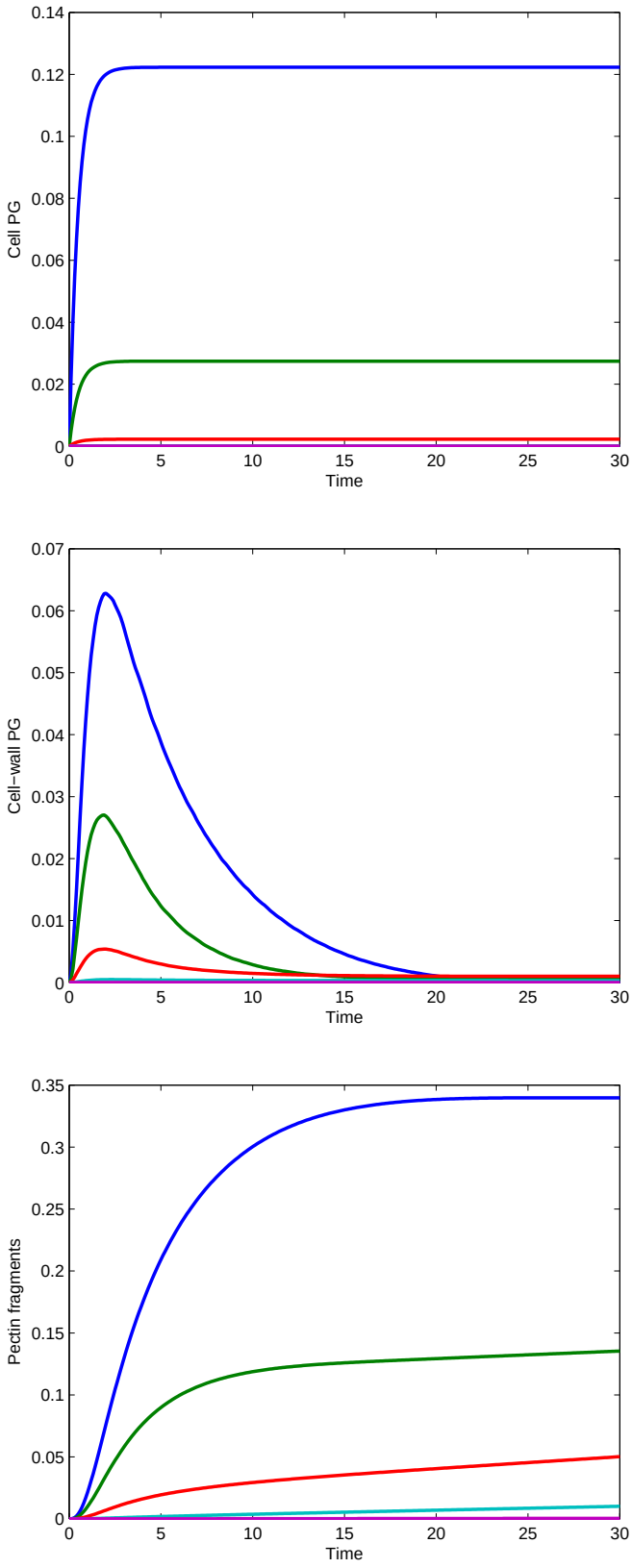

Figure 6: The dependence of P_i, Q_i and R_i on time t for the multiple cell model with 10 cells, calculated with $\mu = 1$, $\alpha^* = 1$, $m = 2$, $R^* = 1/2$, $\gamma = 2$, $\beta = 1$ and k_i given by (8). In each plot, from top to bottom, the curves are for: $i = 5$ and 6; 4 and 7; 3 and 8; 2 and 9; and 1 and 10.

As with any mathematical model, the qualitative behaviour of the solutions should be explored by varying the parameter sets. This task has not been undertaken, although we have made some preliminary suggestions at the end of the previous section. Further, and more importantly, there is a need to determine appropriate values of the parameters involved, or at least a realistic understanding of their size. This experimental undertaking is a matter for further research. Finally, in order to test the model presented here, the numerical results obtained need to be validated against experimental data. Again, these endeavours will form part of future projects in this area.

4.2 Possible extensions

A number of extensions to our mathematical model seem possible, and we discuss these here.

An interesting question not addressed in our mathematical model is how the abscission zone arises in the first place. It seems that the differentiation of cells in these zones occurs in the early stages of the plant's life, and perhaps many months before abscission. This process is evidently important scientifically but is outside the scope of the present modelling exercise.

It appears that the mechanics of abscission is an important component of the process, and is worth further pursuing from a mathematical point of view. It may be that, once the fracture has been initiated, internally generated mechanical stresses cause adjacent cells to separate so that the crack propagates quickly and produces a clean break without damaging the surrounding cells, although their cell walls have also been weakened by the pectin degrading enzymes. A mechanical study of abscission could involve analysing (both through mathematical modelling and experimental work) the residual stresses that build up through the plant's life, and the effect of introducing a weak spot to the structure. Further, there are possibly important issues related to the responses of the abscission cells to mechanical signals, and how this affects the abscission process. Finally, there is a suggestion that abscission cells may expand in the direction perpendicular to the separation layer after cell wall degradation, and that this expansion increases the internal stresses that lead to rupture. It is not clear at present if such an expansion actually takes place and if the expansion contributes to the cell separation or is merely its consequence. These questions deserve further attention.

Another open problem (which is possibly related to the mechanics cited above) is how the vascular tissue, which is immune to degradation by PG, breaks off, and why it does so at the same precise location as the separation layer. Because the vasculature is not degraded, breaking it presumably requires much larger forces than breaking the bonds between cells. A description of this process would require a detailed mechanical model of the breakage, which is beyond the scope of the present study.

As mentioned in Section 3, we do not consider levels of ethylene and IAA in our mathematical model, but instead treat the relationship between the hormones as leading to a trigger. That is, when the balance in favour of ethylene reaches some critical level, we assume the entire abscission process begins. This approach is, of course, rather simplistic, and there seems to be a broader question of the role of these hormones in regulating the timing of abscission that could be analysed with the use of mathematical models. It would be interesting to pursue these ideas, with the goal of coupling the hormone regulation to the production and secretion of PG. A suggestion could be to introduce dependent variables for ethylene and IAA concentrations, with governing equations for each, and make the rate of PG production, k, and the rate of secretion, α, be dependent on these quantities. Relevant issues to consider could include whether IAA reduces the level of ethylene receptors (both inside and outside the abscission zone), and whether ethylene reduces IAA synthesis and transport more generally. Note that avenues for modelling responses

to hormones in plants, based on an analogy with Michaelis-Menten kinetics, are discussed in [15] (and more broadly in the review paper [16]), for example. Further, a detailed ODE-type model for the genetic response in *Arabidopsis* root cells to ethylene signalling is presented in the recent paper [4]. Finally, a simple advection diffusion model for the concentration of IAA is treated in [17], with the goal of making predictions of the location and timing of abscission.

For the purposes of a first model, we have treated the synthesis of PG, and ignored other enzymes that break down pectin in the middle lamella. Perhaps the next important such enzyme is β-1,4-glucanase; the inclusion of the production and effects of β-1,4-glucanase in a future model may be desirable.

4.3 More general models

More generally, cell separation in a plant is important for other processes apart from abscission. Examples of these processes include: dehiscence, where plants deliberately open pods or fruit to release seeds; leaf expansion, where plants increase surface area to capture light and carbon dioxide for photosynthesis; and fruit ripening. Many of the same chemical signals and mechanical process occur in these processes as those described in this document (for abscission), including possible signals such as ethylene and IAA, and also the production of pectin-degrading enzymes. Indeed, the sort of simple mathematical modelling approach we have attempted could easily be adapted to other cell separation processes, and this point should be kept in mind in future studies. Furthermore, similar modelling ideas could be applied to scenarios in which foreign invaders, such as pathogenic fungi, secrete endopolygalacturonases to degrade the cell walls within plants.

Finally, we note that a quite detailed mathematical model has recently been presented in [3] for the abscission process of harvested grapes in storage. The model focuses on the abscission zone at the junction between the pedicel and the berry, and includes the effects of respiration, degradation of cellulose, dissolution of the middle lamella, and water transport. Predictions are made of the "fruit detachment force", which is a measure of the berry adherence strength. Many of the ideas presented in [3] are worth pursuing in future models of organ shedding, more generally.

Acknowledgements: On behalf of all of the project contributors, the report authors wish to thank Professor Jerry Roberts and Dr Zinnia Gonzalez-Carranza for their enthusiasm, wisdom and patience throughout the four days of the study group. We are grateful for all their hard work of the organisers of the study group. Finally, we acknowledge helpful comments and suggestions by Prof. Ming Yang and Dr John Ward.

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