Modelling the likelihood of introduction and establishment in Australia of *Erwinia amylovora* associated with entry of apple fruit from areas with fire blight

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Summary

The biological consequences of introducing *Erwinia amylovora*, fire blight, into Australia are reviewed based on predictive modelling of disease severity in different regions. Current knowledge about carriage and detection of the pathogen on fruit, ineffectiveness of fruit dips, and the likelihood of establishment from imported fruit are discussed in relation to developing predictive models. Three probability models were constructed to determine the effect of major parameters on the probability of detecting an infested orchard, of importing infested fruit and of outbreaks of fire blight from infested fruit. The parameters considered were: reliability of detection methods, size of orchards, sampling rate, infestation level at time of testing, proportion of infested fruit remaining infested at shipping, and potential for disease establishment from infested fruit. Assuming the symptomless carriage of one infested apple in 1,000 in export orchards at time of testing, a sampling rate of one apple in 6,000, 200 million fruit imported each year, and the most realistic estimates available for the other parameters, the predicted probability of failing to detect an infested orchard is 62% and the probability of at least one fire blight outbreak in Australia in 30 years is 78%.

Introduction

Modelling the consequences of introduction and establishment of disease is an everyday action, albeit often undertaken subconsciously, when a set of parameters is examined for their effects either in a local or an exotic disease situation.

Australia is currently free of fire blight, which is caused by the bacterium *Erwinia amylovora* (Burrill 1882) Broadhurst, Buchanan, Rogers and Smith 1920. Recent approaches by various countries to export apples to Australia have resulted in the comprehensive assessment of quarantine risks by Australian quarantine authorities and other concerned organizations. One specific proposal for import of apple fruit from "fire blight free districts of New Zealand" (Anon. 1989) was released for public comment as part of a new and more open quarantine policy (Cook 1988).

The fundamentals of this policy are "to aid safe, efficient production in Australia's plant and animal industries, and the conservation of its flora and fauna, in order to contribute to national economic and social welfare". The stated quarantine objectives include providing protection against unwanted diseases, facilitating safe trade and plant introductions and applying sound scientific principles to decision making. A major requirement for this is biological and economic risk assessment, which is, in essence, predictive modelling. One principle of risk assessment is to adopt a conservative response if scientific or economic information is inadequate, whilst encouraging further research, technology and surveys.

This paper reviews the major biological risks of introduction and establishment of fire blight through importation of infested fruit into Australia. The probability of carriage and detection in fruit imports, the likelihood of establishment of the disease from such fruit, and the determination of the potential severity of fire blight to major pome fruit growing areas by predictive modelling will be discussed.

Modelling the consequences of disease introduction and establishment

As a first step in determining the biological risks of an exotic pathogen, the question must be asked: Is the disease likely to be significant if established? Fire blight is described as one of the most destructive diseases of pome fruit in the world, and also one of the most erratic and unpredictable (van der Zwet and Keil 1979, Reil *et al.* 1979, van der Zwet *et al.* 1988).

In order to determine the likely severity of fire blight in Orange, a major pome fruit area of NSW, Penrose *et al.* (1988) used a predictive model based on published work. The criteria for a fire blight potential infection day (PID) during apple and pear bloom were that the maximum temperature must exceed 18°C on a day when rainfall is recorded, or must exceed 18°C on a day when relative humidity exceeds 70% and rain occurred on the previous day. A multiple infection period (MIP) is two consecutive potential infection days. Luepschen *et al.* (1961) found that the occurrence of one MIP led to serious blossom blight.

At Orange, N.S.W. an average of 6.2 PIDs occurred during blossoming each season over the 12 year period studied, with MIPs occurring in 10 of 12 years, and two or more MIPs in 5 of 12 years. Similar results were found for Batlow, N.S.W. (Penrose, unpublished).

Wimalajeewa and Atley (1990) conducted a similar study in Victoria which found an average 13.5 PIDs per season at Tatura, with two or more MIPs in all of the 11 years studied and five or more MIPs in 9 of 11 years. Heaton (pers. comm.), in Queensland, determined an average of 14 PIDs per season at Applethorpe, with two or more MIPs in five of six years studied and five or more in two of six years.

Roberts (pers. comm.) undertook a comprehensive study of all major pome fruit growing areas in Australia using three different predictive models and concluded that all mainland pome fruit areas would have severe blossom blight in most seasons. Tasmania would experience moderate blight in fewer seasons than the mainland, with rare severe blossom blight.

The overseas predictive models used were not designed for the purpose of determining potential threat in a non-infected area, but rather were empirically evolved to explain historical epidemics (van der Zwet *et al.* 1988) or to time spray applications, and there may be some limitations when the models are extrapolated to new climates. Thomson and Hale (1987) compared fire blight incidence and environment in New Zealand to the western United States, using two predictive models: a less stringent temperature threshold model (Thomson *et al.* 1982) and a model based on temperature maxima and minima correlated to generation time of *E. amylovora* (Billing 1980). The models satisfactorily predicted epiphytic development of the pathogen in flowers, but the incidence of fire blight in New Zealand was less than predicted by both models. Higher populations of competitive saprophytic bacteria, including *E. herbicola,* in blossoms compared with the western United States may be associated with this difference.

This New Zealand study highlights the still unpredictable nature of fire blight and the need to examine other local factors not included in current models. Variations in epiphytic *E. amylovora* populations in California in climatically distinct districts also cannot be accounted for by the temperature threshold model (van der Zwet *et al.* 1988). A wide range of other factors including hail injury, prevalent in many Australian orchard areas, and insect activity have a profound effect on increasing fire blight severity (van der Zwet and Keil 1979).

Factors in modelling risk and establishment

Carriage by fruit

Van der Zwet and Keil (1979) reported that fire blight can spread on fruit, budwood and orchard equipment. They concluded from an extensive literature review that *E. amylovora,* resident in apparently low numbers, can spread through trees systemically to fruit as well as to shoots, roots and flowers. Van der Zwet *et al.* (1990) found, in a trial with the susceptible

cultivar Rome Beauty, that *E. amylovora* could be isolated from internal apple tissue, excluding the calyx and stem regions, only when canker stem lesions were less than 30 cm from the fruit. In contrast however they report presence of the pathogen within the core tissues and the stem and calyx ends of the relatively resistant cultivar Delicious from blight free trees adjacent to infected trees of the cultivar Jonathan.

Mature symptomless fruit have clearly been shown to carry *E. amylovora* (van der Zwet and Van Buskirk 1984, Hale *et al.* 1987, Scholberg *et al.* 1988, van der Zwet *et al.* 1990). Symptomless fruit from apparently healthy orchards have also been shown to carry *E. amylovora* (van der Zwet *et al.* 1990). The protected calyx appears to be the main area of survival rather than the epidermal surface (Hale *et al.* 1987).

Trade in fruit may have brought fire blight to England in the 1950s, with its subsequent spread through Europe by other means of dispersal. However, as with many quarantine outbreaks, the cause will always remain uncertain, as the pathogen may have arrived years before the first reported outbreak in Kent in 1958 (van der Zwet and Keil 1979).

Analysis of the results of a recent study indicate carriage on fruit may be more significant than initially suspected. Van der Zwet *et al.* (1990) cite two cases of apparently healthy fruit reaching export markets in a diseased condition. They investigated various fruit injury and storage conditions on survival and development of *E. amylovora* in harvested apples and found that 1% of apparently healthy Rome Beauty fruit from fire blight free orchards developed a rot symptom caused by *E. amylovora* in storage, compared with 15% of fruit from blighted orchards. In a geographic survey of orchards in four regions of North America the authors found 11% of fruit from blighted orchards carried the pathogen whilst 1% of fruit from apparently blight free orchards were infested. The presence or absence of the pathogen varied with variety and location, but even 1% of fruit from the relatively resistant cultivar, Delicious, from blight free orchards were found to be carriers.

Detection methods

With potential carriage of *E. amylovora* on fruit from symptomless trees and orchards established, reliance must be placed on detection of the pathogen by sensitive laboratory methods in export orchards free of visible fire blight if exclusion of the pathogen on or in fruit is to be assured. The New Zealand proposal (Anon. 1989) requires a DNA probe test on immature fruit in orchards found consistently free of the disease after visual inspection.

The work of Hale *et al.* (1987) suggested symptomless fruit would best be tested at an immature stage for increased sensitivity, as they found population levels in calyces decline as fruit matures. This phenomenon, however, most probably varies with weather and location, as Scholberg *et al.* (1988) observed continued high levels of infestation to harvest.

Unreliability of detection may have led to a false sense of security in previous studies on fruit as a carrier. Scholberg *et al.* (1988) found 100% infestation of mature apples from symptomless trees, adjacent to infected pears in British Columbia, in contrast to earlier studies by Dueck (1974), who failed to detect any *E. amylovora* from blight affected trees in Ontario. Hale *et al.* (1987) found 3% of mature fruit from heavily blighted trees in New Zealand were contaminated with *E. amylovora* but Roberts *et al.* (1989) found no contamination on fruit from a heavily infected orchard in Washington State. In the study cited earlier (van der Zwet *et al.* 1990), the pathogen was detected on fruit from healthy and blighted orchards in Utah and West Virginia but not on fruit from Washington State or Ontario, although only 40 fruit per cultivar were tested from each orchard.

These marked variations in detection may indicate different climatic factors but they also reflect insensitivity in techniques, and in some cases the low numbers of fruit tested. Thomson and Schroth (1976), for example, could detect *E. amylovora* on pear blossoms in 3-4 hours with immunofluorescence compared with 3-4 days using selective media. The findings of Thomson and Hale (1987), discussed earlier, of high levels of saprophytic epiphytes in New Zealand apples, could account for the lower levels of detection by plating. Hale and Clark (1989) claimed detection of 100 bacteria/calyx using a combined plating/DNA hybridization method which partially overcame competition. Development of reliable techniques of high sensitivity rather than reliance on selective plating media, with their range of inherent problems of bacterial competition and toxicity of ingredients, is likely to lead to a far greater appreciation of the carriage of *E. amylovora* by fruit.

Establishment from infested fruit

It is relatively easy to postulate a wide range of mechanisms by which an infested fruit can lead to establishment of disease. Van der Zwet and Keil (1979) have reviewed the disease cycle of the pathogen, its epiphytic and resident phases, its dissemination by a wide range of insects attracted to ooze, pollen or nectar, its dispersal by birds, and its survival in fruit, plant debris, dried ooze or strands.

Discarded fruit or cores are attractive to insect vectors and may be in close proximity to a susceptible host in, for example, a suburban garden or roadside verge. The fruit itself contains viable seeds which may germinate and possibly develop an epiphytic population from contaminated core, calyx or stem tissue, as suggested by an interception in South Africa of *E. amylovora* on pear seed (Hattingh, 1987) or by the recent studies of pathogen carriage and blight development within apple fruit (van der Zwet *et al.* 1990). Volunteer apple seedlings occur widely in roadside verges, especially in eastern tablelands country (Fahy, personal observation) where there is also abundant naturalized hawthorn, a highly susceptible host of fire blight.

Fruit disinfestation

Carriage on fruit presents no danger of fire blight introduction if elimination is possible using bactericidal dips and if there is no systemic resident phase in fruit, from orchards without fire blight symptoms. The persistence of *E. amylovora* in the calyx may, however, afford protection against dips which may actually force infected debris further into the calcine pore as is sometimes experienced with wet mouldy core (Spotts *et al.* 1988).

Recent laboratory scale studies on bactericidal dips to control *E. amylovora* (Scholberg *et al.* 1988; Janisiewicz and van der Zwet 1988) show chlorine dips to be ineffective yet report effective kill with acetic acid and benzalkonium chloride respectively, which could more readily be due to carry over of residue onto test plates rather than action on the fruit. Wimalajeewa and Fahy (unpublished data) have shown that bacteria sensitive to acetic acid are protected in the calyces of apples. Janisiewicz and van der Zwet (1988) used and recommended 1,400 ppm benzalkonium chloride apparently unaware of their own food regulations in the U.S.A. limiting unwashed food dips to 200 ppm.

In contrast to the above studies Roberts and Reymond (1989) evaluated a wide range of fumigants and dips, including acetic acid and benzalkonium chloride and were unable to eliminate *E. amylovora* with any treatment. The best treatment, buffered citric acid, still left 23% of fruit infested. Thus it appears that dips offer no assurance of *E. amylovora* kill, but may be considered to reduce bacterial levels in a predictive model.

Long term risks

It is important to consider the probability of at least one outbreak over more than one year. Pome fruit are perennial crops; an orchard takes about 4 years to reach economic production, and may continue to be cropped for over 20 years more. The risk of an outbreak of fire blight from imported fruit in any one year may be small, but fruit will be imported every year. A small risk in one year may increase rapidly over time to an unacceptably high risk over 10 or 30 years, the life span of an orchard.

Models for the probability of introduction and establishment of fire blight

Simple probability models were constructed to determine the effect of various parameters on the probability of detecting an infested orchard, of importing infested fruit and of an outbreak of fire blight in Australia. The probabilities of failing to detect infested fruit and of at least one outbreak of fire blight were calculated for various values of the parameters of the models. Generally, one parameter was varied at a time to determine its effect, while the other parameters were held constant, at values termed the default values. In each case,

the default values were set at the best estimate available of the value for each parameter.

The models were:

a) Probability of failing to detect infestation in an infested orchard (PF)

$$PF = [PD \times (1 - PI)]^S$$

where PI = proportion of infested fruit at time of sampling, S = number of fruit sampled per orchard which equals the size of orchard times sampling rate, and PD = probability an infested fruit is detected by the detection method.

b) Expected number of infested fruit imported each year (EIF)

$$EIF = N \times PF \times PI \times PS$$

where N = fruit produced for export each year and PS = proportion of fruit still infested at shipping

c) Probability of at least one outbreak of fire blight (PB)

$$PB = 1 - (1 - PO)^{(EIF \times Y)}$$

where PO = probability of outbreak from single infested fruit and Y = number of years

The sampling unit was assumed to be a single orchard. It was assumed that trees are sampled systematically throughout each orchard, with one fruit sampled from every second, fifth or tenth tree. If an infested fruit is detected, all fruit from that orchard is rejected. The default value assumed for orchard size was 8 ha, consisting of 6,000 trees, yielding an average of 600 fruit/tree, and hence 3.6 million export fruit per orchard. The other values used for orchard size were 1.8 and 7.2 million export fruit for each sampling unit. The number of fruit sampled per orchard (S) was calculated by multiplying the size of the orchard by the sampling rate.

The proportion of fruit infested at the time of testing (PI) was varied from 1/85 to 1/10,000 fruit. The default level was 1/1,000 apples (0.1%), which is based on an extrapolation of the findings of Hale *et al.* (1987), as a level unlikely to be detected by visual inspection.

The sampling rate had a default value of 1/6,000 apples, which corresponds to one apple every tenth tree, as nominated in the New Zealand proposal (Anon. 1989). The other rates used were 1/3,000 apples and 1/1200 apples, corresponding to one apple every fifth tree and one apple every second tree.

The probability that an infested fruit is detected (PD) was included as a variable, because no publication has yet shown what level of *E. amylovora* constitutes an infectious unit in fruit or how reliable the detection methods are. Failure to detect *E. amylovora* may simply mean insensitivity in methodology and not absence of infectious units. The default level of 80% reliability represents an optimistic level. The other values used were 100% and 60%.

The number of fruit produced for export (N) was set at 200 million each year. This was calculated by assuming capture of about 15% of the Australian market (based on 1987 Australian production statistics and an average box size of 130 fruit/box); a capture rate targeted by exporters. In this model, when an infested orchard is rejected, the number of fruit actually imported is decreased.

The proportion of fruit still infested at shipping (PS) was also varied, since there is some evidence that the proportion of infested fruit declines as the fruit matures and because bactericidal dips might be used. The default value was 0.4; that is, of the fruit infested at the time of sampling, 40% are still infested at time of shipping. The other values used were 0.8 and 0.1.

There is no information available on the probability that fire blight will become established from a single infested fruit (PO). Therefore, the default value was set very conservatively at 1/1,000,000. The other probabilities used were 1/100,000 and 1/10,000,000.

The probability of at least one outbreak of fire blight in the importing country was calculated for periods of one year, ten years and 30 years.

Other assumptions used in deriving the models were: a fixed number of orchards are tested; the orchards tested produce a total of N fruit for export each year; all orchards have the same proportion PI of fruit infested; the values of the parameters of the models do not vary from year to year or between orchards; an orchard is rejected if infested fruit are detected, and no additional orchards are tested. The probability of detecting that an apple is infested can be assumed to be independent of the level of infestation in the orchard at the low levels of infestation being considered.

Results

The effect of varying the parameters in model (a) on the probability of failing to detect the presence of infestation is shown in Table 1. Even for the default values of the parameters, which were chosen to be conservative and realistic, the probability of failing to detect infestation was 62%. The effect of the parameters on the probability of one or more outbreaks of fire blight in 30 years in the importing country are also shown.

Table 2 shows the effect of varying parameters in models (b) and (c) on the probability of the occurrence of fire blight in the importing country. It is noticeable that the probability of one or more outbreaks in 30 years is significant, even for very low values of the parameters.

Although the probability of an outbreak in one year may be low, the probability increases over time, and may be unacceptably high after 10 or 30 years.

Figure 1 illustrates the effect of three sampling rates on the probability of outbreaks of fire blight for a range of rates of infestation at the time of testing. Higher sampling rates cause a decrease in the probability of outbreaks. However, for a fixed sampling rate, the probability of outbreaks initially increases, then decreases as the infestation rate decreases. The decrease at low infestation rates is due to the small proportion of infested fruit present when an infested orchard is not detected. The decrease at high infestation rates is due to the decreased probability of failing to detect an infested orchard. The number of fruit expected to be actually imported decreases as either the sampling rate or the infestation level increases, because the number of test orchards rejected increases. When the probability of an outbreak of fire blight is low, the number of fruit imported is also low. For example, for an infestation rate of one apple in 1,000 and sampling rates of one in 6,000, one in 3,000 and one in 1200 apples, the number of fruit expected to be actually imported is respectively 124 million, 76.5 million and only 18 million.

Model (b) was expanded to include three levels of infestation. It was assumed that one third of the orchards had no infestation, one third had one in 1,000 apples infested and one third had one in 200 apples infested. The results for three sampling rates are shown in Table 3. Even at a sampling rate of one apple

Table 1. Effect of four major parameters on probability of failure to detect fire blight infestation on apple fruit in export orchards.

Reliability of detection method (%) (apples)	Size of test unit (x 10^6 apples)	Sampling rate (apples)	Infestation Level at time of testing	Probability of failure to detect infestation (%)	Probability of at least one outbreak in 30 (%)
80[a]	3.6[a]	1/6000[a]	1/1000[a]	62	78
100	"	"	"	55	74
60	"	"	"	70	82
80	7.2	"	"	38	61
"	1.8	"	"	79	85
"	3.6	1/1200	"	9	20
"	"	1/3000	"	38	61
"	"	1/6000	1/200	9	67
"	"	"	1/500	38	84
"	"	"	1/5000	91	6

[a] Default values of parameters. As each parameter is varied, these are the values taken by the other parameters. See text for details. Other default values are shown in Table 2.

Table 2. Effect of two major parameters on probability of establishment of fire blight from fruit imported from orchards with 1/1,000 fruit infested at the time of testing [a]

Proportion of infested fruit remaining infested at shipping (%)	Probability of establishment of disease from infested fruit.	Probability of at least one outbreak in:		
		1 year	10 years	30 years
80	1/100,000	63	100	100
40	"	39	99	100
10	"	12	71	98
80	1/1,000,000[b]	10	63	95
40[b]	"	5	39	78
10	"	1	12	31
80	1/10,000,000	1	11	30
40	"	0.6	6	16
10	"	0.1	1	4

[a] Default values used in calculation of probabilities are described in Table 1.
[b] Default values used in Table 1 and Figure 1.

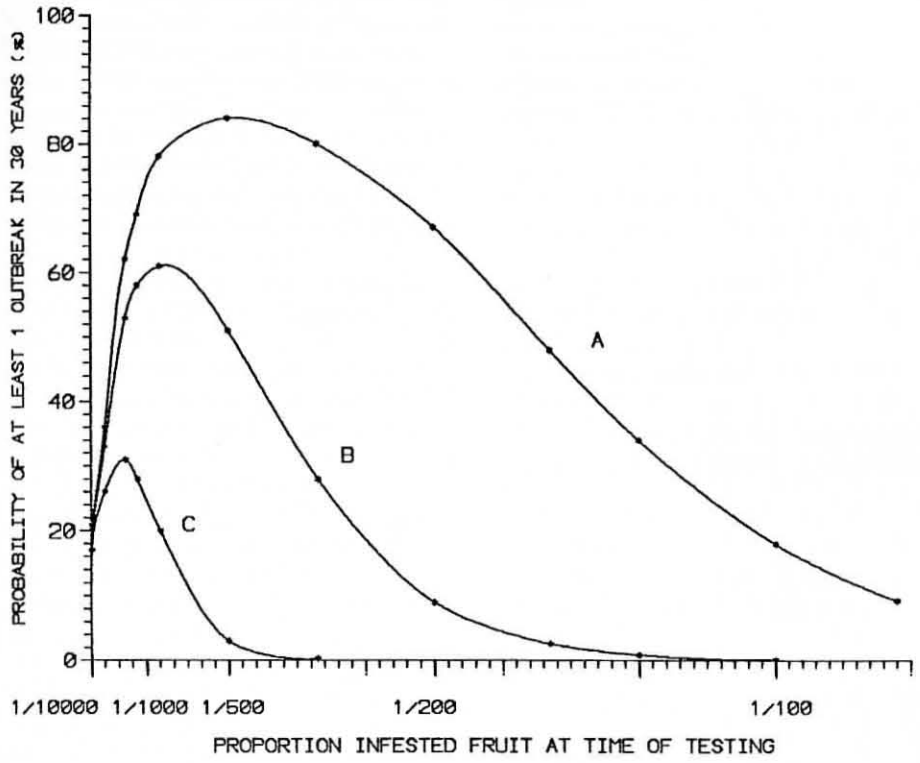

Figure 1. Effect of sampling rate and level of fruit infestation at time of sampling on probability of fire blight outbreaks in the importing country. Sampling rates are: A - 1/6000; B 1/3000; C - 1/1200 fruit sampled. Default values of parameters are shown in Tables 1 and 2. Curves shown are smoothed curves joining points.

Table 3. Effect of sampling rate on expected number of fruit actually imported, and on probability of fire blight outbreak. Infestation levels are 1/3 of orchards - 1/200; 1/3 of orchards - 1/1,000; 1/3 of orchards - nil[a].

Sampling rate (apples)	Number of apples actually imported (million)	Probability of at least one outbreak in 30 years (%)
1/6,000	114	58
1/3,000	93	29
1/1,200	7	7

[a] Default values used in calculation of probabilities are shown in Tables 1 and 2.

in 1200, although the number of fruit expected to be imported is only 7 million, the probability of at least one outbreak of fire blight in 30 years is still 7%. If the number of orchards tested is increased, so that the number of fruit expected to be imported is 200 million, the probability of an outbreak increases to 18%.

The models do not include any allowance for human errors, which may be significant, especially when a large volume of trade is involved. A recent illegal shipment of pome fruit illustrates the danger. This fruit was intercepted in Australia and the calyces were found to contain an *E. amylovora* group organism and abundant populations of viable mites (Fahy, unpublished data), which are vectors of fire blight.

Human errors may occur at any point, from orchard sampling and testing to packing or shipment. Even the very low level of errors likely could substantially increase fire blight risk as fruit is not screened and could come from farms with a very high incidence of disease.

Conclusions

Modelling of the progress of a disease is based on a range of suppositions. The work reported here is a theoretical evaluation of the risk of the introduction, the establishment and the severity of fire blight in Australia. The models used were simple and, of necessity, based on many assumptions. However, the figures derived clearly demonstrate that there are significant risks, even though the risks cannot be fully quantified at this time due to lack of reliable data.

The risks may be more precisely quantified by developing methodologies that lead to reliable, sensitive detection of natural populations of *E. amylovora* on and in fruit, such as DNA amplification. Detection methods must also be capable of detecting all strains of the pathogen. They must be evaluated on naturally infested fruit rather than on fruit artificially inoculated. The methods must be tested under commercial conditions and for low proportions of infested fruit before they are finally accepted.

We have shown that there is a significant risk of the introduction and establishment of fire blight associated with the importation of fruit from a fire blight area despite the precautions of obtaining fruit only from symptomless orchards, rigorous fruit testing and fruit dipping. The question remains, what level of risk is acceptable, considering the pome fruit industry of Australia may be burdened with yet another disease to control, and that there may be adverse effects on exports markets? This requires an economic risk assessment which should be based on sound science and a conservative approach where uncertainty exists.

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