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The development of primary bud necrosis in Thompson Seedless and Flame Seedless grapevines

by

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Die Entwicklung von Nekrosen in den Primärknospen der Rebsorten Thompson Seedless und Flame Seedless

Zusammenfassung: In drei konstant ertragsschwachen Rebanlagen (*Vitis vinifera* Thompson Seedless und Flame Seedless) im San Joaquin Valley (Kalifornien) wurde die Entwicklung von Nekrosen in den Primärknospen untersucht. Die ersten Anzeichen dieser Störung wurden 3—6 Wochen nach der Blüte beobachtet, und sie verstärkte sich innerhalb weiterer 4—6 Wochen. Nach dem Eintritt der Knospenruhe dehnten sich die Nekrosen nicht mehr aus. Die ersten sichtbaren Symptome der Störung waren mehrere Zellschichten dicke, querverlaufende Bänder aus zusammengedrückten Zellen; diese traten an einem oder mehreren Knoten der Knospenachse und an der Knospenbasis auf. Die Zellen in den Zonen der beginnenden Nekrotisierung waren kleiner als die internodialen Zellen und längs zusammengedrückt. Die Nekrose setzte an einem der Bänder zusammengepreßter Zellen ein und schritt akropetal fort. Die Gewebetrennung in der nekrotischen Zone erfolgte durch Zellbruch, nicht durch die Ausbildung eines speziellen Trennungsgewebes. Am häufigsten war die Primärknospe betroffen, gelegentlich konnten aber auch eine oder beide Sekundärknospen nekrotisch sein. In einer anfälligen Rebanlage war die Knospennekrose mit der Beschattung korreliert; in einer schwach gestörten Anlage konnten jedoch Knospennekrosen weder durch Schattierung noch durch Gibberellinbehandlung ausgelöst werden.

Key words: bud, disease, necrosis, growth, histology, light, gibberellic acid.

Introduction

Dormant buds of grapevines are compound buds, each comprised of a large primary dormant bud and two smaller secondary dormant buds. Each of these three buds is a mixed bud containing an entire shoot primordium, and each has the potential to produce flower clusters. However, primary dormant buds are more likely to be fruitful than secondary dormant buds (PRATT 1974). Cluster primordia are differentiated before the bud containing them goes dormant in the year preceding growth, and the number of clusters differentiated is one factor influencing vine fruitfulness.

Primary bud necrosis is another cause of low fruitfulness in many vineyards worldwide. Incidence of bud necrosis varies among cultivars and with viticultural practices. Varieties reported to be sensitive to this disorder include Kyoho (NAITO *et al.* 1986), Queen of the Vineyard (LAVEE *et al.* 1981), Anab-e-Shahi (BINDRA *et al.* 1975), and Thompson Seedless (CHUNDAWAT *et al.* 1977). In these studies, vine vigor (BINDRA *et al.* 1975; LAVEE *et al.* 1981, NAITO *et al.* 1986), high rates of fertilization (BINDRA and CHOHAN 1975; CHUNDAWAT *et al.* 1977), and the use of GA (ZIV *et al.* 1981; NAITO *et al.* 1986) were correlated with high levels of bud necrosis. LAVEE (1987) suggested that high levels of endogenous GA may be involved in inducing high rates of bud necrosis in vigorous vines.

The work described here investigated the timing of bud necrosis and development of the symptoms of this disorder in three vineyards with a history of unfruitfulness. An experiment was designed to determine the effect of GA on the induction of bud nec-

rosis in normally fruitful vines. Because light exposure has been identified as a factor influencing bud break and bud fruitfulness (MAY *et al.* 1976; HOPPING 1977), an additional experiment was designed to examine the effect of shading on the induction of bud necrosis in a normally fruitful vineyard.

Materials and methods

Buds were sampled in 1987 from a chronically unfruitful 8-year-old vineyard of *Vitis vinifera* L. cv. Flame Seedless on Royalty rootstock. Bud samples were taken in 1988 from two chronically unfruitful Thompson Seedless vineyards. The unfruitful vineyards produced less than half as many clusters per cane as fruitful vineyards in their respective areas pruned to the same numbers of buds. One of the vineyards sampled in 1988 was an 8-year-old table grape vineyard on its own roots, trained to a 4-wire slant-top trellis, treated with gibberellic acid (GA) and trunk girdled every year. The second was a 7-year-old wine and raisin vineyard on its own roots, trained to a double-T trellis, which received only a thinning spray of GA at bloomtime each year. Buds were also sampled from an adjacent fruitful vineyard of the same cultivar receiving the same management practices. All of the sampling locations were commercial vineyards of high vigor in the southern San Joaquin Valley of California. Samples of 6 entire shoots were taken from sun-exposed and shaded parts of the vines at 2—4-week intervals. Buds from the basal 15—20 nodes of each shoot were dissected under a microscope and rated visually for cluster development and for signs of bud necrosis.

Representative buds from the basal 10 nodes were embedded in plastic for more detailed observation of the development of primary bud necrosis. Bud scales and hair were removed with forceps, and the buds were fixed in 4 % glutaraldehyde in 50 mM sodium cacodylate, pH 7.2. Samples were rinsed in buffer, then dehydrated in 2,2-dimethoxypropane (DMP), followed by acetone. Samples were infiltrated with glycol methacrylate (Dupont-Sorvall), then polymerized under vacuum on ice to slow the polymerization. Samples were microtomed at 3 μm on a Sorvall JB-4 microtome, using a glass knife. Sections were stained with either toluidine blue in 50 mM sodium acetate buffer, pH 4.5, or with periodic acid-Schiff's reagent (PAS; JENSEN 1962) and counterstained with analine blue black in 7 % acetic acid.

In 1988 experiments were established to test the effectiveness of shoot shading and of GA application in inducing bud necrosis in normally fruitful vines that had not received GA treatments the previous year. Two vigorous, but fruitful Thompson Seedless vineyards adjacent to the unfruitful winegrape vineyard and receiving the same management practices were chosen for the trials.

The gibberellin trial had 3 replicates of 6 vines each. Both treatment and control vines received a bloomtime thinning spray of 10 ppm GA (Pro-Gib, Abbott). The treatment vines received two additional sprays of 100 ppm GA at 9 and 17 d after bloom, which correspond to the times that California table grape growers apply GA to increase fruit size in these varieties.

The shading trial paired one well exposed vine with one shaded vine, with buffer vines on either side of the pair. There were 3 replicates of 5 vine pairs each, for a total of 15 vines in each treatment. The treatments were imposed 3 weeks after bloom. Shoots of the sun exposed treatment were positioned along the foliage wires to provide maximum exposure to the shoots. Shoots of the shade treatment vines were tied to the foliage wires, then shoots from adjacent vines were positioned to cover the experimental shoots, providing one to two leaf layers of shade along the length of each experimental shoot.

Three entire shoots per treatment were analyzed for incidence of primary bud necrosis on each sampling date, for a total of 45 buds per treatment. Buds from all nodes on a shoot were dissected under a microscope and rated on a 4 point scale from completely healthy to completely dead.

Results

Primary bud necrosis occurred during the period between bloom and the onset of bud dormancy in all of the three chronically unfruitful vineyards sampled in this study. Bud necrosis was evident 3 weeks after full bloom in two of the vineyards studied. By this time about 25 % of the buds sampled from the Flame Seedless vineyard, and about 14 % of the buds sampled from one of the Thompson Seedless vineyards had developed symptoms. In both of these vineyards the incidence of bud necrosis increased until 9 weeks after bloom, when dead primaries were found in 85 % and 53 % of the Flame Seedless and Thompson Seedless compound buds examined, respectively. In the other Thompson Seedless vineyard, bud necrosis developed during the period between 6 and 10 weeks after bloom, at which time 72 % of the compound buds examined from this vineyard contained necrotic primary buds. There was no further increase in the incidence of primary bud necrosis after the onset of bud dormancy in any of the three vineyards studied.

The incidence of primary bud necrosis was higher in the basal buds than in the more distal buds on a shoot, and necrosis occurred earlier in the basal positions. Data for one of the Thompson Seedless vineyards is shown in Fig. 1; the distribution of bud

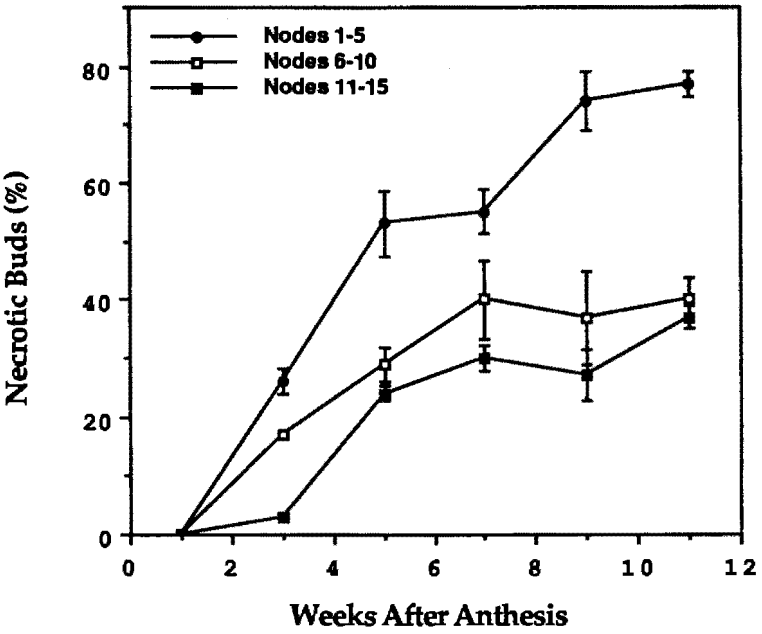
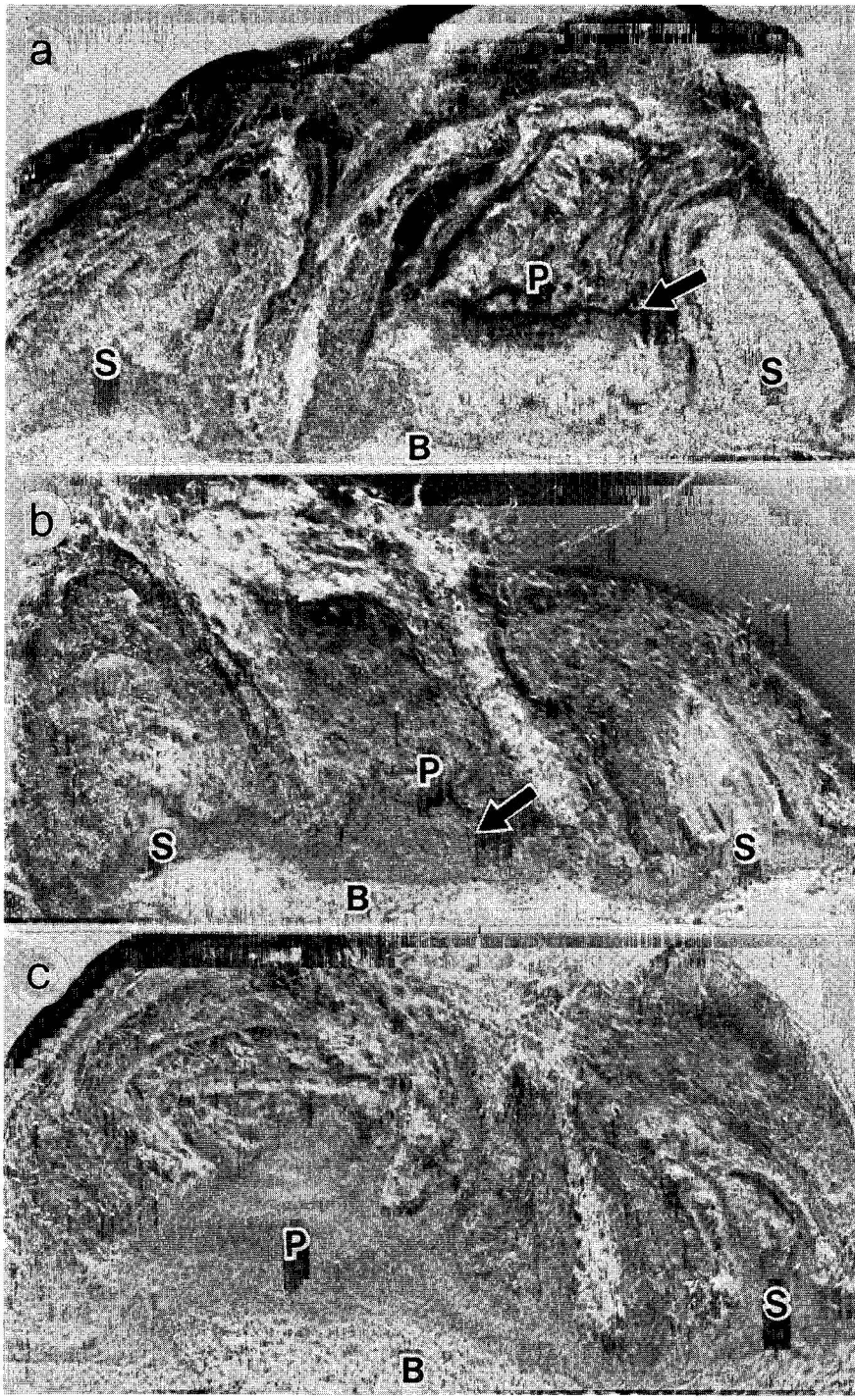


Fig. 1: Incidence of primary bud necrosis at different positions along shoots of Thompson Seedless vines. Each point represents the mean of 30 individual buds. Standard error bars refer to $P < 0.05$.

Auftreten von Nekrosen in den Primärknospen von Thompson Seedless in Beziehung zur Knospenposition. Jeder Punkt stellt den Mittelwert aus 30 Einzelknospen dar. Die Balken geben die Standardabweichung für $P < 0{,}05$ an.


a
P
S
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B
b
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c
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necrosis along the shoot was similar in the other two vineyards studied (data not shown). The mean incidence of primary bud necrosis at the onset of bud dormancy was 78 % in buds at nodes 1—5, 40 % in nodes 6—10 and 38 % in nodes 11—15. Although the patterns of both bud fruitfulness and bud necrosis were highly consistent among shoots, within individual primary buds there was not a correlation between cluster differentiation and bud necrosis ($P < 0.05$). At any node position the incidence of primary bud necrosis was the same for buds that had differentiated clusters as for buds that had differentiated only tendrils.

Microscopic examination showed that the pattern of necrosis was variable (Fig. 2). Some buds died from the base, others from 1 to 4 nodes above the base, and in some, only the apical nodes of the bud died, with up to 5 or 6 healthy nodes below the necrotic area. In the case of partial bud necrosis, the lateral bud immediately below the necrotic zone was frequently larger and further developed than was typical for a lateral bud at that position.

The first visible symptom of incipient bud necrosis was the appearance of transverse bands of compressed cells a few cell layers thick at one or more nodes on the bud axis (Fig. 3 a), and frequently at the base of the primary bud as well (Fig. 5 a). The cells in these bands were smaller than the surrounding cells, and were usually longitudinally compressed and irregularly shaped (Fig. 4). The cell walls in the longitudinal plane often had the appearance of being crushed.

Primary dormant buds in the early stages of necrosis contained almost no starch within the primary bud or in the base of the compound bud (Fig. 5 a). Healthy buds from the same vines had an abundance of starch grains, both in the bud itself and in the base of the compound bud (Fig. 5 b).

Soon after the formation of the incipient necrotic zones, the primary bud died and became completely dry. Bud death proceeded acropetally from one of the zones of incipient necrosis (Fig. 6). The necrosis could begin at any of the zones of compressed cells, and in many of the buds observed the base of the bud remained alive after the distal portion of the bud died. Occasionally, the necrotic zone did not extend laterally across the entire bud, in which case part of the bud became necrotic, while adjacent tissue remained alive (Fig. 6 a).

In many of the buds observed, cell separation at one of the incipient necrotic zones preceded bud death. Separation was due to cell breakage, rather than to the formation of an abscission zone (Fig. 7). The cell breakage occurred either in one layer of cells or in more than one cell layer.

When primary bud necrosis occurred soon after bloom, the two secondary dormant buds enlarged to fill the space occupied by the dying primary bud. When death occurred later in the season, the secondary dormant buds remained small. Necrosis of the primary bud did not affect the fruitfulness of the secondaries within that compound bud (data not shown). Although the primary dormant bud was most often affected, one or both of the secondary dormant buds occasionally also became necrotic.

Fig. 2: Unstained, free-hand sections of the compound dormant buds of Thompson Seedless. a) Partially necrotic primary bud with a dead apex and healthy basal nodes (120 ×). b) Primary bud dead from the base (120 ×). c) Healthy bud (140 ×). Arrows indicate zones of necrosis. B = base of compound bud, P = primary dormant bud, S = secondary dormant bud.

Ungefärbte Handschnitte der zusammengesetzten Winterknospen von Thompson Seedless. a) Teilweise nekrotische Primärknospe mit abgestorbener apikaler und gesunder basaler Hälfte (120 ×). b) Von der Basis her abgestorbene Primärknospe (120 ×). c) Gesunde Knospe (140 ×). Die Pfeile weisen auf nekrotische Zonen hin. B = Basis der zusammengesetzten Knospe, P = dormante Primärknospe, S = Sekundärknospe.

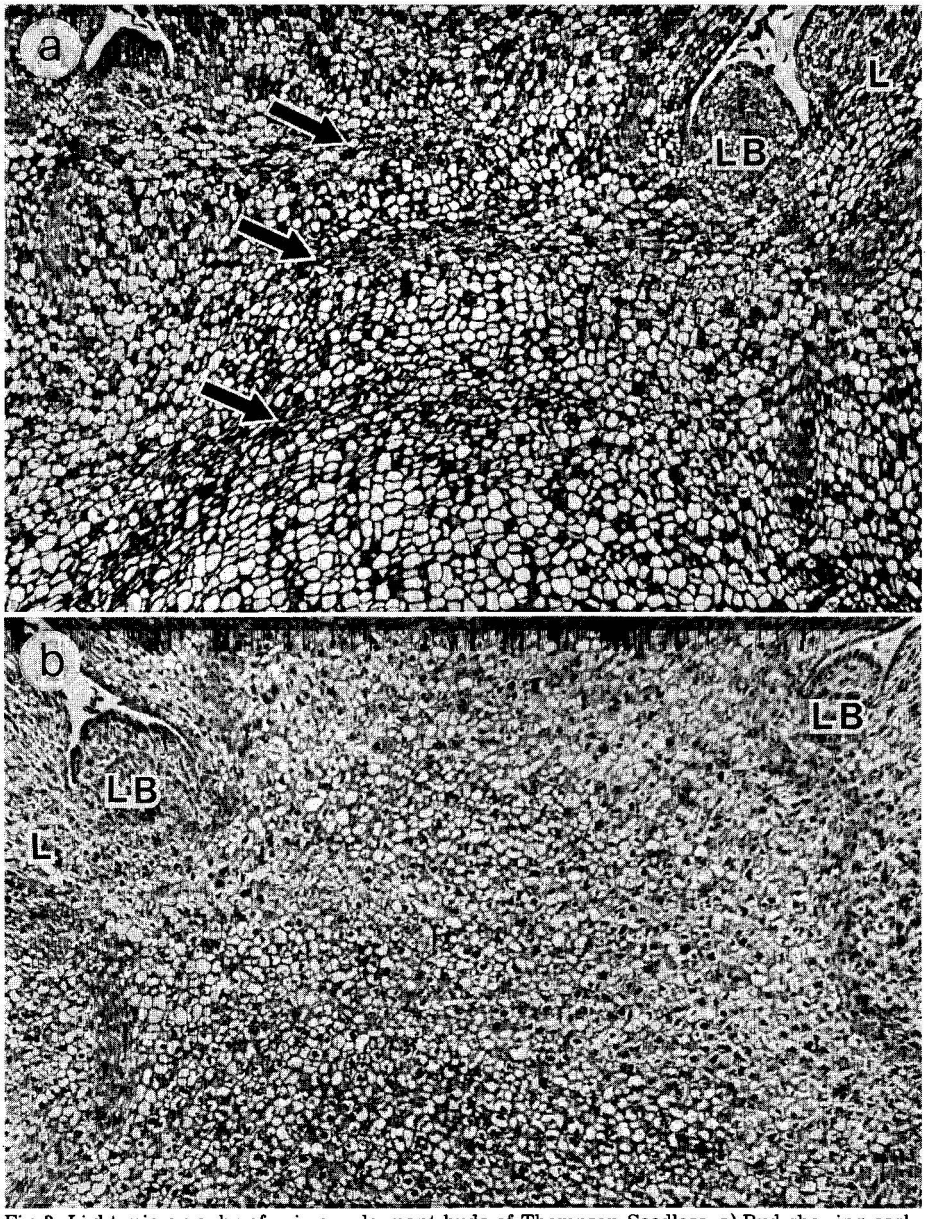

Fig. 3: Light micrographs of primary dormant buds of Thompson Seedless. a) Bud showing early symptoms of necrosis (260 ×). b) Healthy bud (240 ×). Arrows indicate zones of incipient necrosis. L = leaf, LB = lateral bud.

Lichtmikroskopische Aufnahmen dormanter Primärknospen von Thompson Seedless. a) Knospe mit ersten Nekrosesymptomen (260 ×). b) Gesunde Knospe (240 ×). Die Pfeile weisen auf die Zonen der beginnenden Nekrotisierung hin. L = Blatt, LB = Axillarknospe.

Fig. 5: a) Base of primary bud showing signs of incipient necrosis; starch grains are absent (460 ×). b) Base of healthy primary dormant bud with abundant starch grains (460 ×). Arrow indicates necrotic zone. B = base of compound bud, P = primary dormant bud.

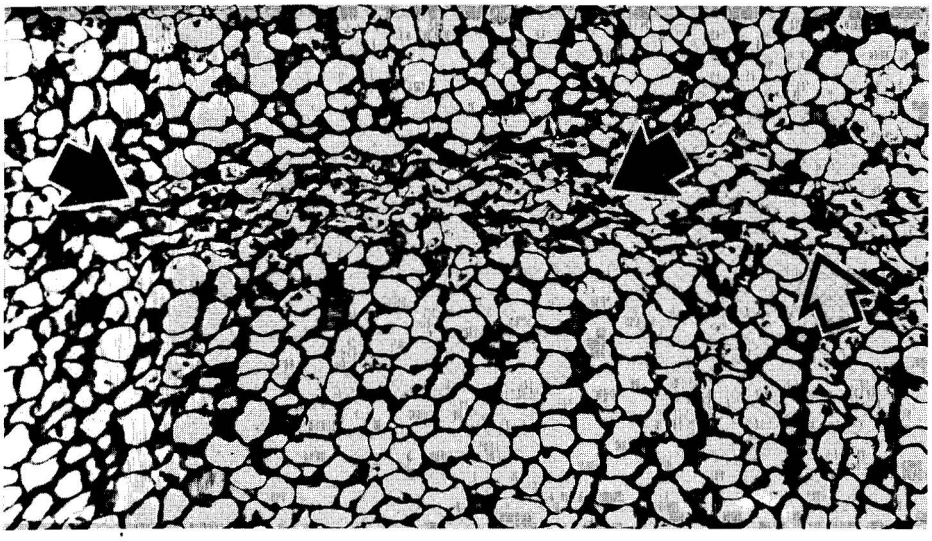

Fig. 4: Zone of incipient necrosis (at arrows) showing longitudinally compressed cells (400 ×).
Zone der beginnenden Nekrotisierung (Pfeile) mit längs zusammengedrückten Zellen (400 ×).

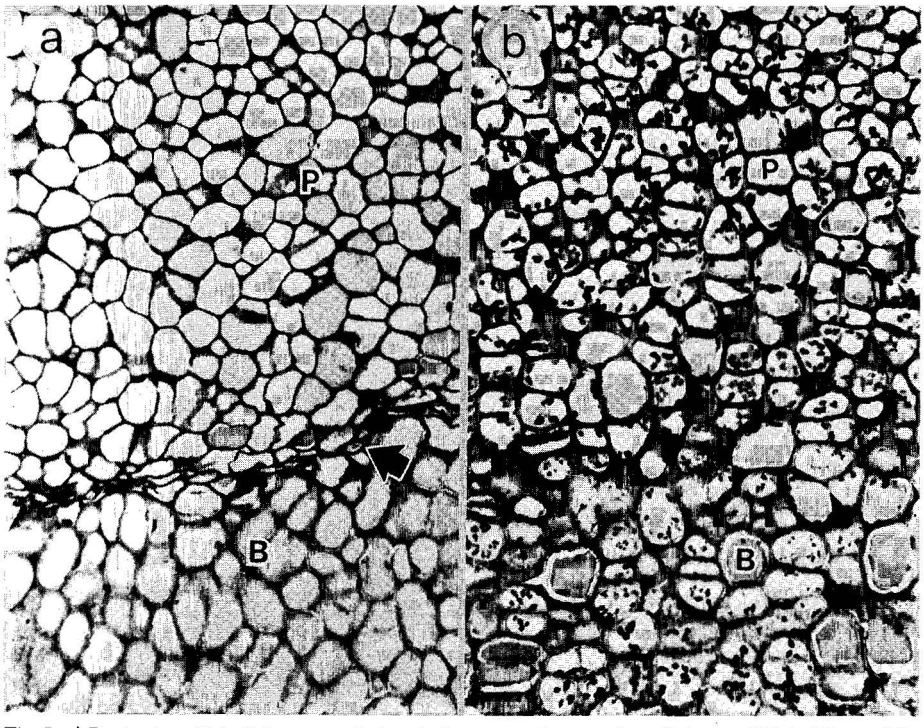

Fig. 5: a) Basis einer Primärknospe mit den Anfangssymptomen einer Nekrose; Stärkekörner fehlen (460 ×). b) Basis einer gesunden Primärknospe mit zahlreichen Stärkekörnern (460 ×). Der Pfeil weist auf die nekrotische Zone hin. B = Basis der zusammengesetzten Knospe, P = dormante Primärknospe.

Effects of shading or GA treatment on bud necrosis and on cluster differentiation · Numbers within a column followed by the same letter are not different at $p < 0.05$

Einfluß von Beschattung oder Gibberellinsäurebehandlung (GA) auf die Knospennekrosen und Infloreszenzdifferenzierung · Untereinanderstehende Zahlen mit demselben Buchstaben unterscheiden sich nicht signifikant voneinander ($p < 0{,}05$)

Treatment	Necrotic buds (%)	Clusters per bud
Unfruitful vineyard		
Sun-exposed	43 b	0.6 b
Shaded	64 a	0.5 b
Fruitful vineyard		
Sun-exposed	12 c	0.8 a
Shaded	16 c	0.7 a
Fruitful vineyard		
GA control	6 d	0.6 b
GA treated	8 d	0.5 b

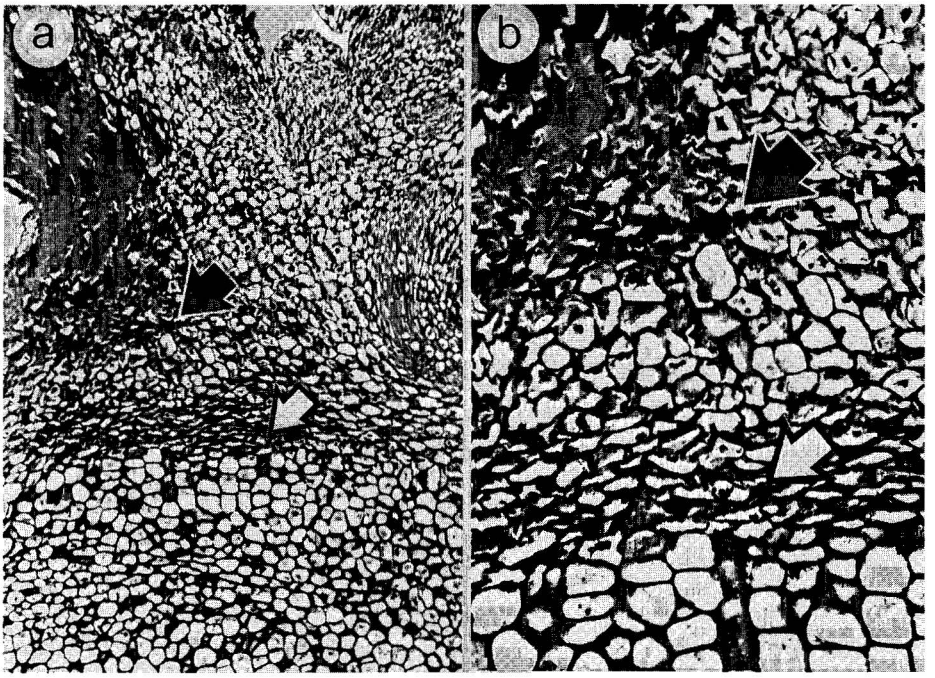

Fig. 6: Death of the tissue cut off by a necrotic zone. Although there is a zone of compressed cells near the base (white arrow), bud death is occurring above the second necrotic zone (black arrow). a) The leaf and lateral bud on the right side of the bud have not been affected by the tissue necrosis on the left side (290 ×). b) More enlarged detail from (a) (720 ×).

Durch die nekrotische Zone abgeriegeltes und abgestorbenes Gewebe. Obwohl nahe der Basis eine Zone zusammengedrückter Zellen vorhanden ist (weißer Pfeil), stirbt das Knospengewebe oberhalb der zweiten nekrotischen Zone ab (schwarzer Pfeil). a) Das Blatt und die Axillarknospe rechts im Bild sind noch nicht von der linksseitigen Nekrose betroffen (290 ×). b) Ausschnittvergrößerung aus (a) (720 ×).

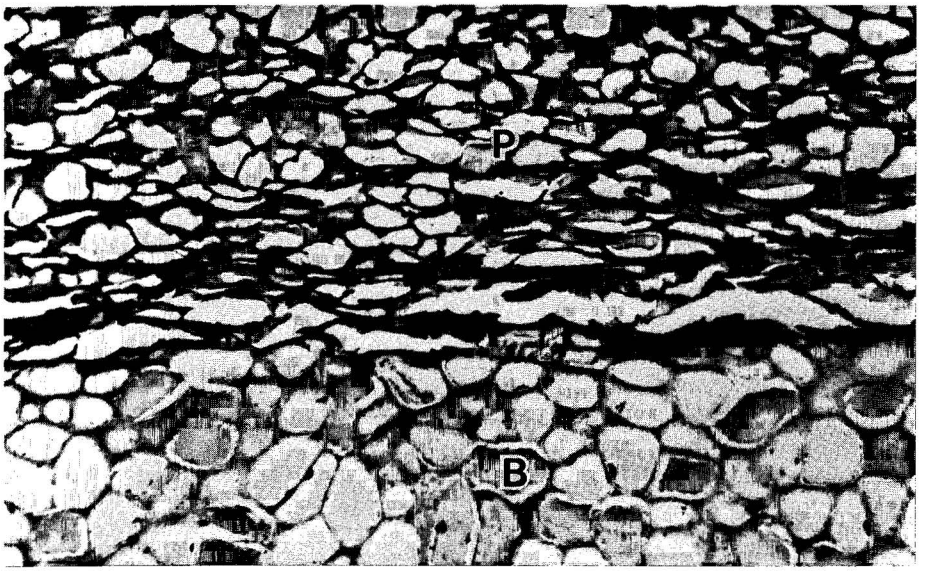

Fig. 7: Lysigenous separation at the necrotic zone at the base of a primary dormant bud (600 ×). B = base of compound bud, P = primary dormant bud.

Abtrennung durch Gewebeauflösung in der nekrotischen Zone an der Basis einer Primärknospe (600 ×). B = Basis der zusammengesetzten Knospe, P = dormante Primärknospe.

The incidence of bud necrosis was low (< 20 %) in the fruitful vineyards that were used for the experimental treatments. However, the patterns of occurrence were the same as in the unfruitful vineyards. Primary bud necrosis was seen only in the basal nodes (numbers 1—8 from the base), and was not seen above node 8 in the fruitful vineyards, although mechanical damage or insect damage were occasionally noted.

Shoots developing in natural canopy shade in the unfruitful vineyards had a higher incidence of primary bud necrosis than those developing in exposed positions on the vine. Data for the vineyard adjacent to the experimental trials is shown in the table. Shading shoots at fruit set, just prior to the appearance of symptoms in the adjacent unfruitful vineyard, did not induce bud necrosis in the normally fruitful experimental vineyard (Table). Shading at this time also had no effect on cluster number. GA treatment also had no effect on bud necrosis in the healthy experimental vineyard adjacent to the unfruitful vineyard (Table). The two experimental vineyards differed from each other in incidence of bud necrosis and in mean cluster number, but within each vineyard the experimental treatments were not significantly different at $P < 0.05$.

Discussion

The timing of the first appearance of primary bud necrosis was similar to that reported by LAVEE *et al.* (1981) for Queen of the Vineyard. In that cultivar necrosis occurred only during a 2-week period starting 3 weeks after bloom, while in Thompson Seedless and Flame Seedless the incidence of necrosis continued to increase over a 4—6-week period, which began between 3 and 6 weeks after bloom.

The higher incidence of primary bud necrosis in the basal buds is in agreement with the observations of LAVEE *et al.* (1981) and NAITO *et al.* (1986). Although a negative correlation has been noted between the fruiting potential of a bud and the incidence of necrosis based on bud position (LAVEE *et al.* 1981), we found no significant correlation between fruitfulness and necrosis in individual buds. Many of the dying buds had differentiated clusters before becoming necrotic.

Although the necrotic lesions usually originated at a node along the bud axis, the specific node at which this occurred was variable. The development of lesions began in bands of compressed cells, which developed at several nodes as well as at the base of the bud. Tissue separation at these necrotic zones was lysigenous, without the formation of a defined abscission layer. Occasionally buds were observed in which one side of the bud was completely dead, and the other apparently healthy, which was also observed by BINDRA and CHOHAN (1975). However, the more common pattern was that reported by LAVEE *et al.* (1981), in which all of the tissue above the necrotic zone became completely dry, while the tissue below that node remained healthy. When the primary bud died from the base early in the season, the secondary dormant buds expanded to fill the space left by the dying primary, which is consistant with the observations of LAVEE *et al.* (1981). However, expansion of the secondaries was not seen when the primary bud died later in the season, or when there were surviving lateral buds on the healthy basal portion of a partially necrotic primary bud. The occasional death of one or both of the secondary buds reported here was also observed by BINDRA and CHOHAN (1975).

The lack of starch grains in buds showing signs of incipient necrosis suggests either that these buds are not strong sinks for photoassimilates or that necrotic buds have an altered carbohydrate metabolism. BAINS *et al.* (1981) similarly found that fruitful vines of moderate vigor accumulated higher concentrations of starch in growing shoots and mature wood than did overly vigorous vines, which have a higher incidence of bud necrosis (BINDRA and CHOHAN 1975; NAITO *et al.* 1986). However, if carbohydrate depletion is assumed to be the cause of bud death, it is difficult to explain the apparently healthy state of the basal nodes of many necrotic buds. The lack of starch in necrotic buds may well be the result of altered metabolic processes during senescence, rather than the cause of bud death.

The failure of GA application to induce bud necrosis in a fruitful Thompson Seedless vineyard is in contrast to the effects of GA on Kyoho reported by NAITO *et al.* (1986). However, Thompson Seedless is less sensitive to GA-induced bud death than many cultivars (WEAVER and MCCUNE 1961). ZIV *et al.* (1981) found that although GA increased bud necrosis in Queen of the Vineyard when applied before or soon after anthesis, it had no effect when the application was made well after bloom. The lack of response seen here is therefore probably due to a combination of the relative insensitivity of these cultivars and to the timing of the sizing sprays at 9 and 17 d after bloom. These results suggest that the commercial practice of later GA applications to increase berry size probably does not contribute greatly to the problem of bud necrosis in healthy vineyards of these cultivars.

We observed a correlation between the incidence of bud necrosis and the location of a shoot in the shaded interior or exposed exterior of vines prone to this disorder. Other researchers have seen similar correlations (J. PEREZ, personal communication). The failure of shoot shading to induce bud necrosis in the non-susceptible vineyard suggests that this is probably not the major cause of bud necrosis, although shading appears to be an aggravating factor in vineyards liable to this disorder. The correlation between vine vigor and bud necrosis reported by many authors (BINDRA *et al.* 1975;

LAVEE *et al.* 1981; NAITO *et al.* 1986) is therefore probably due to effects of vigor other than increased canopy shade.

Summary

The development of primary bud necrosis was studied in three chronically unfruitful vineyards of *Vitis vinifera* L. cvs Thompson Seedless and Flame Seedless in the San Joaquin Valley of California. Evidence of this disorder was first seen between 3 and 6 weeks after bloom, and increased in severity over a period of 4—6 weeks. Incidence of bud necrosis did not increase after the onset of bud dormancy. The first visible symptom of this disorder was the appearance of transverse bands of compressed cells a few cell layers thick at one or more nodes on the bud axis and at the base of the bud. Cells in these zones of incipient necrosis were smaller than the internodal cells and were longitudinally compressed. Necrosis started at any of the bands of compressed cells and proceeded acropetally. Cell separation at the necrotic zone was due to cell breakage, rather than the formation of an abscission zone. Although the primary bud was most often affected, one or both of the secondary buds were sometimes also affected. Although shading was correlated with a higher incidence of bud necrosis in susceptible vineyards, neither shoot shading nor GA application induced necrosis in a vineyard with a low incidence of this disorder.

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