한국식물병리학회지 11(1) : 9-16(1995)
Korean J. Plant Pathol. 11(1) : 9-16(1995)

Control of Apple Valsa Canker by Localized Spraying with Neoasozin Solution, an Arsenic Fungicide

Jae Youl Uhm* and Hyung Rak Sohn[1]
Department of Agricultural Biology, College of Agriculture, Kyungpook National University, Taegu 702-701, Korea
[1]Kangwon Rural Development Administration, Chuncheon, Korea

네오아소진의 국부처리에 의한 사과나무 부란병의 방제

엄재열* · 손형락[1]
경북대학교 농과대학 농생물학과, [1]강원도 농촌진흥원

ABSTRACT : Undiluted neoasozin solution (6.5% a.i.), an arsenic fungicide, was sprayed on 169 cankers of apple trees from early March to September in 1987 twice at intervals of one week without scraping off the affected barks. Among the treated cankers, 79.9% ceased to grow within 1~7 weeks, 13.0% showed partial development, and 7.1% grew continuously to girdle the branches. The partially developed cankers, however, could also be cured by an additional spray after slightly piercing at the edge of cankers to facilitate the penetration of the chemical. When the canker growth was blocked, cankers were encircled by cracks developed at the marginal area of the cankers. If the cracks developed once, very few cankers grew beyond them. The above results suggest that the crack development may be the consequence of the host defense activity to wall off the pathogen. In addition to the curative efficacy, the neoasozin solution inhibited sporulation of the pathogenic fungus almost completely. However, the pathogen survived for more than three months in some cankers that externally appeared to be cured, suggesting that an indirect mode of action of the chemical against apple Valsa canker seems to be still more persuasive than the direct fungicidal effect. In the final examination conducted in the mid April of the next year, 72.7% of the cankers were completely cured by the two successive neoasozin treatments. Moreover the cure rate became 83.1% if that of partially developed cankers which were also completely cured by an additional treatment was also taken into account. Since 1989 when this method was widely applied in apple orchards in Korea, apple Valsa canker has been effectively controlled to reach a tolerable level.

Key Words **: apple Valsa canker, chemical control, neoasozin solution.**

Apple Valsa canker caused by *Valsa ceratosperma* (Tode ex Fr.) Maire has been one of the most important limiting factors of apple production in Korea (1, 2) as well as in Japan (6). From the late 1960s the epidemic of the disease greatly increased, and the loss due to the disease reached its highest peak in the 1970s, when a large number of apple trees had been cut down. A survey made by the present authors in the vicinity of Taegu city in 1985 revealed that more than 60% of adult trees were diseased. In spite of these serious epidemics, no reliable control measures have been developed yet. The surgical treatment, removal of the cankered bark and painting with a fungicide, was the main control measure at that time. This method, however, not only requires much labor but also has defect of resumption of growth. The method could not be fully practiced due to the shortage of labor in rural areas. In these situations we tried to find a curative fungicide which might be labor saving.

In the previous paper (8), we reported how we reached the finding to use neoasozin solution, ammonium salt of ferric methyl arsenic acid, for the control of apple Valsa canker and the potentials

*Corresponding author.

for the control of the disease by localized spraying of the chemical on the canker without laborious scraping off the cankered bark.

The rationale for applying the neoasozin solution was based on the mechanism of infection by the fungus and that of the mycelial expansion within the host tissue. The causal fungus produces several kinds of necrotic toxins by enzymatic degradation of phloridzin that is specifically contained in the tissue of *Mallus* sp. (3, 4). The toxic substances were assumed to be responsible for the necrosis which is the characteristic symptom of the disease caused by this fungus (3, 4, 7). In a histopathological study, Tamura and Saito (7) have shown that a zone of browning or necrosis was invariably observed in advance of mycelial growth, and have also assumed that the toxins are the cause of it. Combining these two facts, it can be said that the necrotic toxins take part not only in symptom development but also in mycelial invasion into the healthy tissue. These aspects led us to an assumption that the inhibition of the toxin production, which can be achieved by inhibiting the extracellular enzymes of the fungus involved in the phloridzin degradation, may bring about the block of the canker growth.

In efforts to find out some substances that can block the canker expansion through the inhibition of enzymes, several heavy metal compounds ($CuSO_4$, $PbCl_2$, and $HgCl_2$) and an arsenical ($AsHNa_2O_4$) were tested by spraying the aqueous solution on the cankers. Among them, $AsHNa_2O_4$ showed a remarkable inhibitory effect on the canker expansion (8). Good results were obtained in the subsequent trial by using the neoasozin solution which is the only arsenic pesticide under current use in Korea against rice sheath blight (8).

The neoasozin solution was registered for apple Valsa canker in Korea, and has been widely used since 1989. At present, the disease has been reduced to a tolerable level in Korea. In this paper we report on the properties of neoasozin solution for the control of apple Valsa canker.

MATERIALS AND METHODS

Chemical treatment. From March to September in 1987, 169 cankers with various sizes formed on the trunks or branches were selected from three apple orchards in the vicinity of Taegu city in Korea. The boundary of the cankers was marked with oil pens prior to the chemical treatment. Undiluted neoasozin solution (Sungbo Chemical Co. Ltd., Seoul, Korea) was locally sprayed twice at intervals of one week on the cankers without scraping off the affected barks. In spraying the chemical, attention was paid to insure the solution should be covered 5 to 10 cm beyond the canker boundary, in accordance with the canker size.

Examination of the course of canker curing. The canker growth after the chemical treatment was examined by measuring the length of the cankers at one week intervals until they ceased to expand. The time of crack development at the margin of the cankers was also examined.

Examination of fungal survival in the cankers that ceased to expand. Among 86 cankers treated with the chemical from March to mid April, 50 cankers which were blocked to expand within 6 weeks were selected and cankered tissue fragments were taken at 42, 65 and 93 days after the first treatment. Small fragments of diseased tissue were laid on PDA plates as aseptically as possible, and the survival of the pathogenic fungus was determined by the growth of the fungus on the plates.

Examination of sporulation on the neoasozin treated cankers. Formation of pycnidia and extrusion of spore-horns were examined on 139 cankers treated with the neoasozin solution from March to June.

Examination of final cure rate. The cankers which were estimated to be cured or blocked to expand for more than 3 months during the year of treatment were examined to detect any possible resumption of expansion in the mid April of the next year, the time when the cankers usually grow actively.

RESULTS AND DISCUSSION

Blocking of the canker growth. In the examination conducted up to 49 days after the first treatment, 135 out of the 169 cankers ceased to grow within the examination period, while 22 partially expanded and 12 grew continuously to girdle the branches (Table 1). There were two types of partial expansion. One type was that only a part of the canker continuously expanded, and the other was that the growth was once blocked for a considerably long time but resumed growth at a certain marginal

Table 1. Inhibition of canker growth in apple Valsa canker by the localized treatment with neoasozin solution

Date of 1st treatment	No. cankers	No. cankers ceased to grow								No. cankers partially grown		No. cankers continuously grown	Average[c] growth after 1st treatment (mm)
		Days after 1st treatment											
		7	14	21	28	35	42	49	Total	I[a]	II[b]		
Mar. 9	22	1	0	5	1	3	4	2	16	4	2	0	24.7
Mar. 21	27	3	5	3	10	1	—	—	22	4	0	1	20.6
Mar. 30	22	2	4	1	5	2	5	—	19	1	0	2	19.0
Apr. 11	15	7	1	2	2	1	—	—	13	1	0	1	10.7
Apr. 28	14	5	5	2	0	—	—	—	12	1	1	0	13.2
May 27	9	3	4	1	—	—	—	—	8	1	0	0	16.2
Jun. 16	20	6	3	6	2	0	—	—	17	0	2	1	16.7
Jul. 9	14	7	1	1	0	0	1	—	10	0	2	2	13.0
Sep. 12	26	10	4	3	1	0	—	—	18	0	3	5	15.0

[a]Cankers of which only a part was continuously expanded without ceasing.
[b]Cankers partially resumed to grow after ceasing for a considerably long time.
[c]Growth of cankers which ceased to grow within the examination period.

portions of cankers (Table 1). Those cankers, almost without exception, were formed on the branches having smooth skin on which a wax layer was well developed. The cause of the partial expansion may be due to the insufficient penetration of the chemical into the bark tissue, since most of those cankers could be cured by an additional treatment with slightly piercing at the margin of the canker to facilitate the chemical penetration (Fig. 1). However, 3 cankers of which the partial expansion took place transversely across the branch failed to be cured by the additional chemical treatment, since those cankers girdled almost whole branches when their recurrence was found (Table 5).

The cause of the continuous growth exhibited by 12 cankers cannot be explained decisively. However, one theory can be conceivable. Browning of the xylem under the healthy bark outside of the canker lesion can frequently be observed when scraping off the affected bark in the spring (6), and sometimes it extended to more than several ten centimeters beyond the cankers (6). If the chemical did not fully cover well beyond the range of browned xylem, those cankers might be continuously expanded, since the chemical does not kill the pathogen in the bark tissue as will be described later in this paper. Therefore, the chemical should be applied much wider than the areas of the cankers to obtain a high cure rate.

The canker growth was not blocked immediately after the chemical treatment. Fig. 2 shows the canker expansion at one week intervals after the chemical treatment. The days required for the blockage of the canker growth was quite variable (Table 1); however, it usually took shorter time to block the canker growth in the cankers treated with the chemical in the growing season of the apple tree than those treated in the dormant season. More than one half of the cankers treated from April onward ceased to grow within 14 days, while those treated in March took longer time to be blocked (Table 1). When the chemical was treated in September, 10 out of 26 cankers ceased to expand immediately after the chemical treatment. The canker growth in this disease usually ceases during the hot season of July and August, sometimes accompanying crack development between the diseased and healthy tissue, but resumes to expand when it becomes cool in September (6, 7). In early September when the 1st chemical treatment was done in our experiment, some of the growth in some cankers still suspended and the spontaneously developed cracks during the summer were still discernible. In these conditions, the canker growth might be immediately blocked by the 1st chemical treatment.

The canker expansion after the chemical treatment for a certain period of time may be explained by the invariable development of the degenerated zone in advance of hyphal tip by the toxic substances. Since the neoasozin solution cannot kill the

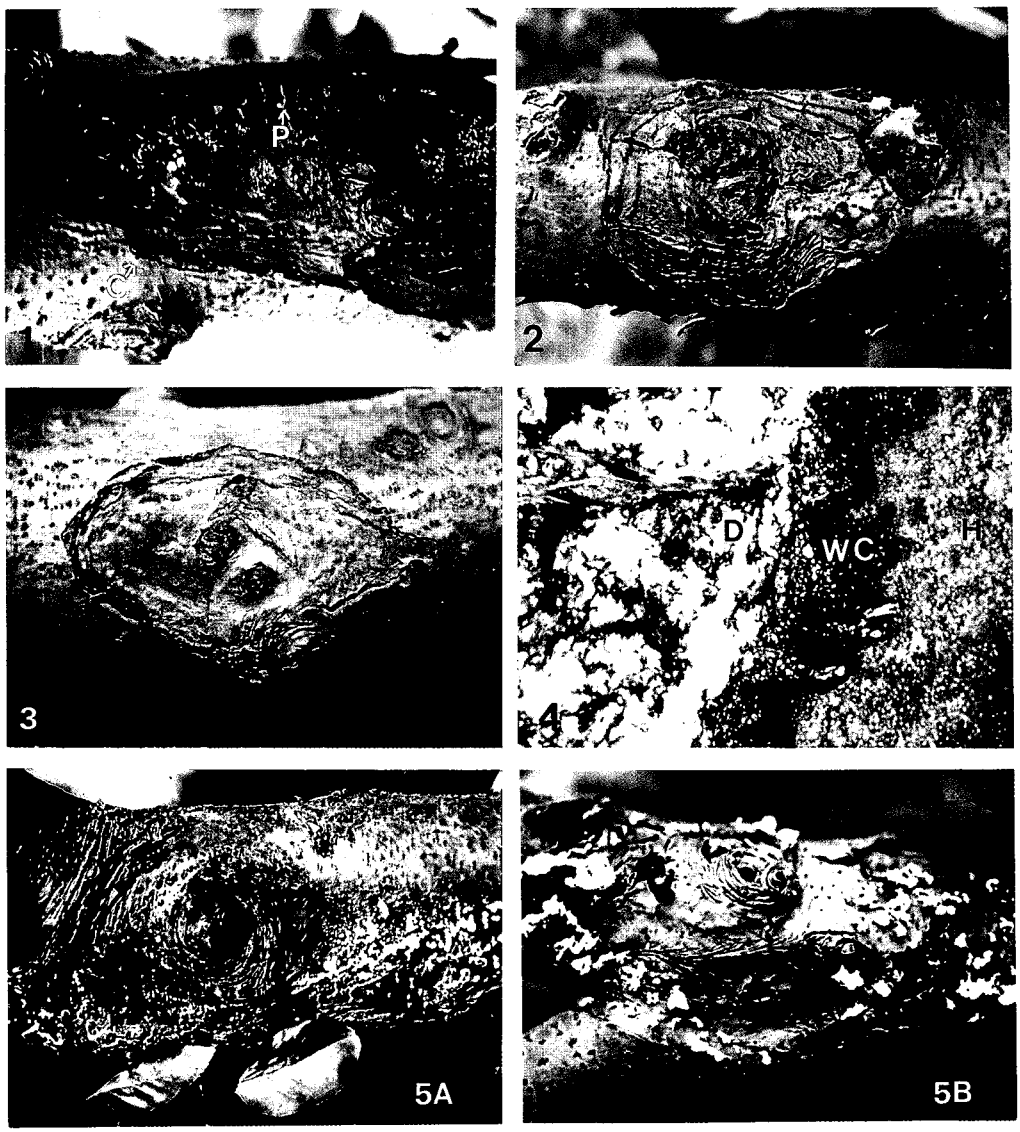

Fig. 1~5. 1. Crack development on the partially grown canker by the additional treatment with slight piercing at the margin of the canker to aid the penetration of the chemical; P : Piercing; C : Crack. **2.** Canker growth after the treatment with neoasozin solution. The inner most circle is the canker boundary at the time of the 1st treatment. **3.** Crack development at the periphery of the canker treated with neoasozin solution. **4.** Wound cork cell development at the healthy side of the crack; H : Healthy tissue; D : Diseased tissue; WC : Wound cork cells. **5.** Colonization of saprophytes on the cankers treated with neoasozin solutions; A : *Trichoderma* spp; B : *Schizophyllum commune.*

mycelia but inhibit the production of toxic substances (8), the mycelia may continuously grow after the chemical treatment through the degenerated zone, leading canker expansion for a certain period of time after the chemical treatment. In this respect, the variable ranges of canker expansion after the chemical treatment as shown in Table 1 might be the reflection of the differences in degenerated zones of the cankers.

Crack development. Once the cankers ceased to grow, they were dried and hardened, and the cracks developed at the margin of the cankers (Fig. 3). The

Table 2. Crack development at the periphery of the cankers ceased to grow after the treatment with neoasozin solution on apple Valsa canker

Date of 1st treatment	No. cankers		No. cankers with crack development								
	Treated	Ceased to grow	Days after the 1st treatment								
			0	14	21	28	35	42	49	56	Total
Mar. 9	22	16	0	0	0	0	0	1	13	1	15
Mar. 21	27	22	0	0	0	1	19	1	0	0	21
Mar. 30	22	19	0	0	0	0	15	2	0	0	17
Apr. 11	15	13	0	0	0	12	1	0	0	0	13
Apr. 28	14	12	0	2	9	1	0	0	0	0	12
May 27	9	8	0	4	4	0	0	0	0	0	8
Jun. 16	20	17	0	11	4	1	0	0	0	0	16
Jul. 9	14	10	0	7	1	1	0	0	0	0	9
Sep. 12	26	18	6	5	0	4	0	0	0	0	15

crack development can be used as an indicator for estimating the cure of the cankers as already reported in our previous paper (8), since cankers seldom grew beyond the cracks. However, cracks were not always developed on the cankers which ceased to grow, especially not discernable on the cankers formed on the trunks or scaffold limbs of old trees of which the bark tissues were extremely thick.

A consistent tendency was found in the time for the crack to develop. For the cankers treated with the chemical on March 9, the cracks mostly appeared at about 49 days later, but 35 days later for those treated on the 21st and 30th of March (Table 2), indicating that the cracks appeared almost simultaneously around late April or early May in spite of 12 or 21 day difference among the three treatments conducted during March. When the cankers were treated in April, the time for the crack development became even shorter, 28 and 21 days on those treated on the 11th and 28th of April, respectively. It eventually took 14 days for the crack development on the cankers treated in late May onward.

The fact that the cracks developed almost simultaneously from late April to early May on the cankers treated at different dates during March, and that much shorter period of time was required for the crack development on the cankers treated afterwards suggests that the crack development may be related to the metabolic activity of the apple tree. Based on the time of crack appearance, it can be said that the apple tree had reached a certain state of metabolic activity in late April or early May needed for developing the cracks. When the plant reached its full metabolic state around late May, the crack development might become more rapid, taking only about 14 days. However, the cracks appeared on the cankers treated in September may be different in the state of crack development from those on other cankers treated in different periods of time. As shown in Table 2, natural cracks usually appear during summer (6, 7) were still discernible at the time of the first treatment on the six cankers among those treated in September. Since no more canker growth was ever observed in those cankers, the unstable natural cracks might have developed into the stable ones by the neoasozin treatment, which might lead to the cure of the cankers.

The detailed mechanism of the crack development has not been elucidated as yet; however, two aspects which might be responsible for the crack development were observed in this experiment. A thick layer of cork cells was developed on the healthy side of the cracks immediately after their formation (Fig. 4). When the diseased bark was peeled off about one week after the crack formation, growth of the callus from healthy tissue was observed as we already described in our previous report (8). The detailed histopathological study on the cankers treated with neoasozin solution has been conducted in our laboratory and will be published in a subsequent paper.

Survival of the pathogen within the cankers that ceased to expand. The pathogenic fungus survived in 78, 48 and 32% of the cankers until 42, 65 and 93 days after the first treatment, respectively (Table 3). The cankers in which the fungus survived until

Table 3. Survival of *Valsa ceratosperma* on the cankers treated with neoasozin solution

Date of 1st treatment	No. of cankers examined	No. of cankers with the pathogen isolated		
		42[a]	65	93
Mar. 9	13	10	6	4
Mar. 21	17	14	9	6
Mar. 30	10	7	5	4
Apr. 11	10	8	4	2

[a]Days after the 1st treatment.

65 or 93 days after the 1st treatment were almost cured at least in an external view. These results suggest that the chemical does not kill the pathogen directly, and thus canker growth is not promptly blocked after the chemical treatment. Survival of the pathogen within the cured canker suggests the indirect mode of the chemical action as we initially postulated. That is the arsenic contained in the chemical inhibits the extracellular enzymes of the pathogen from degrading the phloridzin into the toxic substances which may denature the healthy tissue prior to the fungal invasion and thus help the fungal hyphae advance into the host tissue. However, this postulation has not been demonstrated as yet.

As the days went on, some of the cankers became colonized by *Trichoderma* spp. (Fig. 5A) or by *Shizophyllum commune* Fr. (Fig. 5B). The surviving pathogenic fungus may have been suppressed by these or other microorganisms. In the cankers on which no sign of colonization by other microorganisms was noted, the pathogenic fungus might have been annihilated by dryness.

Sporulations on the cankers treated with the neoasozin solution. Even though the neoasozin solution cannot kill the pathogenic fungus within the host tissue, it was turned out that the chemical inhibits the sporulation of the fungus almost completely. Among the 129 cankers treated with the neoasozin from March to June, sporulation was observed on three cankers only. Relatively small numbers of pycnidia were formed on 16 cankers just beneath the bark surface, but spore horns were never extruded during the experiment (Table 4). Those pycnidia were eventually destroyed by other microorganisms such as *Trichoderma* spp. or *Shizophyllum* spp., or fell away with dead barks. Inhibition of the pycnidium formation was observed not only on the ceased cankers but also on the partially and continuously growing cankers. Even on the cankers of active sporulation, similar inhibitory effects were also noted. Extrusions of spore horns were observed on the five cankers, three treated on April 28 and two on May 27, at the time of the first chemical treatment, but the extrusion was completely ceased by the treatment. Probably the inhibition property of the neoasozin on the sporulation of the pathogenic fungus might have played an important role in the drastic reduction of apple Valsa canker since 1989 when the chemical treatment was widely applied in apple orchards in Korea.

Table 4. Effect of neoasozin solution on the sporulation of *Valsa ceratosperma* on the canker

Date of 1st treatment	No. of cankers examined	No. of cankers	
		Pycnidia formed	Spore-horns extruded
Mar. 9	22	6	2
Mar. 21	27	1	0
Mar. 30	22	7	1
Apr. 11	15	2	0
Apr. 28	14(3)[a]	0	0
May 27	9(2)	0	0
Jun. 16	20	0	0

[a]Number of cankers from which the spore-horns extruded at the time of the 1st treatment with neoasozin solution.

The final cure rate. It was difficult to define the complete cure of the cankers within the year of chemical treatment, because the cracks which can be used as an indicator for estimating the cure of cankers were not always discernible, and moreover, the pathogen survived for a long period of time within the cankered tissue. Therefore, the treated cankers were reexamined in mid April of the next year. Table 5 shows the curative efficacy of the neoasozin solution obtained in the year of chemical treatment and the subsequent year. Among the 169 cankers treated from March to September, 126 cankers were estimated to be cured within the year of chemical treatment. In addition to them, 19 partially expanded cankers on which the cracks also developed by the additional treatment, and the nine cankers which were checked for more than three months but no cracks developed were also temporarily regarded to be cured (Table 5).

Table 5. Curative efficacy of neoasozin solution on apple Valsa canker

Date of 1st treatment	1987				1988			
	Number of cankers				Number of cankers			
	Growth ceased		Growth not ceased	Total	Complete cure	Growth resumed	Missing[a]	Total
	Cracked	Not cracked[b]						
Mar. 9	15(4)[c]	1	0(2)[c]	22	14(3)[c]	0	2(1)[c]	20
Mar. 21	21(4)	1	1	27	19(3)	1	2(1)	26
Mar. 30	17(1)	2	2	22	18(1)	0	1	20
Apr. 11	13(1)	0	1	15	11(1)	0	2	14
Apr. 28	12(2)	0	0	14	9(1)	1	2(1)	14
May 27	8(1)	0	0	9	6(1)	2	0	9
Jun. 16	16(2)	1	1	20	13(2)	2	2	19
Jul. 9	9(2)	1	2	14	10(2)	0	0	12
Sep. 12	15(2)	3	5(1)	26	12(2)	5	1	20
Total	126(19)	9	12(3)	169	112(16)	11	12(3)	154

[a] Cankers missed during the experiment.
[b] Cankers ceased to grow for more than 3 months but no cracks developed.
[c] Figures in the parenthesis of this table are the partially expanded cankers on which an additional treatment was done with slight piercing at the margin of the canker.

In the examination conducted in April of the next year, 128 out of the 154 suppressed cankers in the previous year were estimated to be completely cured because no additional growth was noted thereafter on (Table 5). Only 11 cankers resumed growth in the next spring. The other 15 cankers including three partially expanded cankers in the previous year were missing during the experiment. From the above results, it was thought that the complete cure of the cankers treated with the neoasozin solution can only be determined in the next spring. The exact cure rate could not be calculated due to the missing cankers. If the missing cankers and 16 partially cured cankers are excluded from the total number of treated cankers, the cure rate is estimated to be 72.7%. If the 16 partially expanded cankers which were also completely cured by an additional treatment were included in the total canker number, the cure rate increases to 83.1%.

요 약

유기비소계 농약인 네오아소진은 사과나무 부란병에 대해 탁월한 방제효과를 나타내었다. 1987년 3월부터 9월까지 169개의 자연 발병한 병반에 네오아소진 원액을 병환부를 도려내는 등의 전처리 없이 1주간 간격으로 2회 병반 부위에 국부 처리한 결과, 79.9%의 병반은 1~7주 이내에 진전이 정지하였고, 13.0%의 병반은 부분적으로 진전했으며 7.1%의 병반은 계속 진전하였다. 부분 진전된 병반에 약제의 침투를 돕기 위해 병반의 주변부에 칼로 자상을 입히고 약제를 다시 처리한 결과, 대부분의 병반 진전이 정지되었다. 진전이 정지된 병반의 주의에는 균열이 생기게 되며, 일단 균열이 생기면 병반이 더 이상 진전되는 경우는 거의 없었다. 약제의 처리로부터 균열 발생까지의 소요 시간에 있어서는, 3월 중에 약제 처리시는 4월 하순부터 5월 상순에 걸쳐 병반에 균열이 생겼고, 그후는 약제의 처리로부터 균열 발생까지의 소요 일수가 점차 줄어들어 4월 하순 이후의 처리에서는 2주 이내에 균열이 생겼다. 위 결과로 볼 때, 균열의 발생은 기주의 대사활동과 관련된 것으로 생각된다. 또한 네오아소진은 병반의 치유뿐만 아니라 포자형성을 강하게 저지하였다. 그러나 살균효과는 낮아서 조직 속의 균사는 완치된 병반에서조차 장기간 생존하고 있었다. 약제를 처리한 다음해의 4월 중순에 전년도에 처리한 병반을 재조사한 결과, 2회 약제처리에 의한 완치율은 72.7%였으며, 2회 처리후 부분적으로 진전된 병반에 추가로 약제를 처리하여 완치된 병반을 포함시키면 완치율은 83.1%에 달했다. 1989년부터 이 방법이 실제 포장에서 광범위하게 적용된 이래 부란병에 의한 피

해는 크게 줄어들게 되었다.

REFERENCES

1. Kim, M. H and Lee, J. T. 1974. Ecological studies on the outbreak of apple canker. *Research Review of Kyungpook National Univ.* 18 : 91-98.
2. Kim, S. C., Won, C. N., Lee, E. K., Son, J. S. and Han, E. D. 1970. Studies on the canker of apple tree caused by *Valsa mali* Miyabe et Yamada. *Korean J. Plant Prot.* 9 : 81-84.
3. Koganezawa, H. and Sakuma, T. 1982. Possible role of breakdown products of phloridzin in symptom development by *Valsa ceratosperma. Ann. Phtopath. Soc. Japan* 48 : 521-528.
4. Natsume, H., Seto H. and Otake, N. 1982. Studies on apple canker. The necrotic toxins produced by *Valsa ceratosperma. Agric. Biol. Chem.* 46 : 2101-2106.
5. Sakuma, T. and Takakuwa, M. 1983. Apple Valsa canker in Japan : Historical review. *Shokubutsu boeki* 37 : 227-230.
6. Sakuma, T. and Tamura, O. 1983. Apple Valsa canker in Japan : Mechanism of infection and canker development. *Shokubutsu boeki* 37 : 239-240.
7. Tamura, O. and Saito, I. 1982. Histopathological changes of apple bark infected by *Valsa ceratosperma* (Tode ex. Fr.) Maire during dormant and growing periods. *Ann. Phytopath. Soc. Japan* 48 : 490-498.
8. Uhm, J. Y. and Sohn, H. R. 1991. Neozin solution, a possible control agent of apple Valsa canker. *Ann. Phytopath. Soc. Japan* 57 : 577-581.