

Elucidation of Cause of Cotyledon Black-Decay of Soybean Sprout by Bean Bug, *Riptortus clavatus*

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ABSTRACT Cotyledon Black Decay (CBD) on soybean sprout mimics the black spot due to microbial infection. CBD, not visible or predictable at seedlot state, for some reason, shows up exclusively on cotyledon of soybean sprout during sprouting process. Such an incidence rate fluctuated from 0.8 to 19.5% over three years from 2004. We suspected some pod-infecting anthracnose fungi and/or pod-blight pathogen, or pod-sucking bean bug, one of the major pests of soybean, might have involved, of which we ruled out fungal pathogen because it was preventable through heat treatment, a proven method for seedlot disinfestation. The healthy seeds artificially fed by bean bug for one to seven days were sprouted, and 6 to 41% of the soybean sprout revealed the CBD mimic to those occurred in soybean sprout from previous commercial seedlot screening experiments. This finding is the first report to confirm that bean bug damage to pod at R₈ stage is directly responsible for the CBD, which did not concur with any other deleterious effects on sprouting such as reduction in hypocotyls elongation and rooting except unsightly sprout quality. However, earlier feeding either at green pod or greenish yellow pod stage (R₆ -early R₇ stage) resulted in rather severe damages, which strikingly reduced hypocotyls growth to about one fourth to about two third, as well as the reduction in rates of seed germination.

Keywords : soybean sprout, cotyledon, black-decay, bean bug, *Riptortus clavatus*

The soybean sprouts have been one of the prominent vegetables for a long time from ancient times in Korea. In recent years, the market of soybean sprouts was estimated to seventy hundred billion won domestically (Kang *et al.*, 2005).

The great barrier to produce healthy soybean sprouts were black rot on hypocotyls of sprout by *Colletotrichum* sp. and other bacterial rots, which could be prevented using heat treatment method developed recently by our research team (Lee *et al.*, 2007a, b). Interestingly enough, the cotyledon black-decay (CBD) is not visible or recognizable at seedlot stage until it shows up exclusively on cotyledon upon sprouting, which was not controlled by the heat treatment method. Thus, it's causal agents were not elucidated so far, because no other microbiological agents were found responsible for it. Therefore, one last possibility was that the CBD might have been resulted from the insect damage, so we inspected bean bug, *Riptortus clavatus* (Hemiptera: Alydidae), a major pod pest of soybean in the field in Korea.

Jung *et al.* (2005) reported that the podding stage (R₃) was most susceptible to bean bug feeding, resulting in empty pods and completely underdeveloped seeds and full seed stage (R₄) was also susceptible, resulting in deformed seeds and distinctly injured seed coats. Even in the most recent reports by Oh *et al.* (2007), who worked on the damages by stink bugs of soybean sprouts, the relevancy of bean bug damage in soybean sprouts CBD has not yet been addressed.

In this paper, we report our attempts to elucidate the cause of CBD of soybean sprouts. So the authors present the precise results that are experimentally reproducible.

MATERIALS AND METHODS

Investigation of insect damage in soybean (Pungsannamulkong) seedlots

Seedlot collections produced in Jeju Island from 2004 to 2006 were screened for the CBD of soybean sprout by culturing the soybean sprouts from sample of each seedlot.

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One liter of soybean seeds each were cultured to soybean sprouts through the small scale sprouting system for seven days at $22\pm 2^{\circ}\text{C}$, of which one hundred sprout samples were inspected for each treatment among 5 replications.

Artificial feeding for CBD reproducibility

The healthy and dried soybean seeds were subjected to feeding by bean bug, ten each seeds were fed by 10 bean bugs in the small cages for 7 days. The experiments were carried out for 10 replications. After feeding, the samples were observed under microscope and cultured to sprouts in small scale system in order to confirm the reproducibility of the syndrome.

Influence of sucking periods by bean bug and seed maturity on the incidence of CBD

Tests were carried out by subjecting 10 seeds each to feeding by 10 bean bugs for 1, 3, 5, and 7 days, respectively in the small cages with 10 replications. The treated samples were also cultured to soybean sprouts, which were recorded for the incidence of the syndrome, the germinating rate, length of hypocotyls and roots. The incidence of CBD and growth, quality of sprouts were inspected. To determine the affect of soybean seed maturity on the susceptibility to bean bug, seed samples at green-pod (R_6 stage), greenish yellow-pod (R_7 stage) and yellow-pod (R_8 stage), respectively, were exposed to sucking by bean bugs. Then, the soybean seeds were grown to sprouts to inspect the seed germination, CBD and sprout growth. The experiment was done with 3 pods of soybean per 10 bean bugs for 7 days in small cages with 5 replications.

Statistical analysis

For all experiment, data analyses were performed using the SAS statistical software (V 9.1, SAS Institute Inc.). Significant differences in treatments were determined using Student's t test as $P = 0.05$.

RESULTS

Fluctuation of CBD from the commercial seed-lots over the years

Incidence of CBD of soybean sprouts were ranged from

0.8 to 19.5% from the commercial seed-lots under storage, which were produced in 2004 to 2006 in Jeju Island as shown in Fig. 1. For those soybean seedlots produced in 2004, the CBD occurred exceptionally at higher rates 19.5%. The annual difference in CBD incidence was attributed to some variable factors associated with that specific year. We suspected some pod-infesting bean bug, one of the major pests of soybean, might have something to do with. The results obtained by feeding healthy seeds with bean bug were shown in Fig. 2.

The bean bug could puncture through the hard soybean seed easily with stylet and sucked up some nutrients. The stereomicroscopy of bean bug damage on the seed surface is shown as holes in Fig.2A & B. The trace of stylet sheath of bean bug on the corresponding site on surface of soybean seeds were also observed distinctly under SEM (Fig. 2C & D).

Influence of feeding periods for sprouting on the development of CBD incidence

The fully matured seed sucked by bean bug were sprouted, which revealed the CBD on the soybean sprout as shown in Fig. 3. The symptom of cotyledon black decay was described as follows: The CBD symptom on the soybean sprouts appeared initially after 5 hours imbibition of the seeds in water before germination. The dark brown irregular type



Fig. 1. Annual fluctuation of cotyledon black-decay developed on the soybean sprouts from the seedlots produced over the three years in Jeju Island. One liter of soybean seedlot samples sprouted for 7 days by watering the container for 3 minutes so as to overflow for 30 seconds at every three hour at $22\pm 2^{\circ}\text{C}$. Bars corresponding to different letters differ significantly at $p = 0.05$.

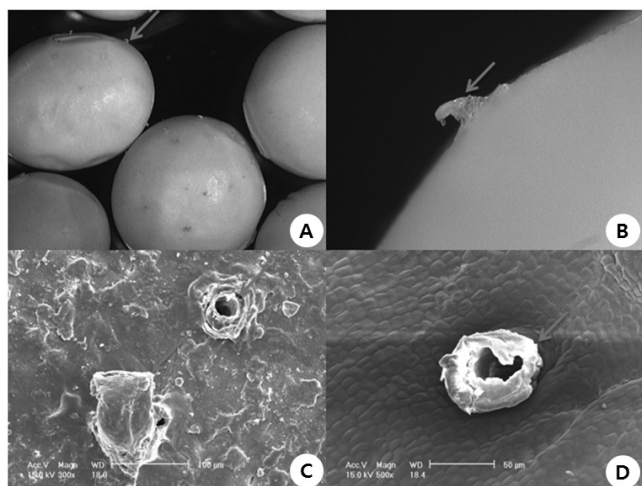


Fig. 2. The stereoscopy (2A & B) and scanning electron microscopy SEM (2C & D) of bean bug damage on the seed surface and trace of stylet sheath of bean bug attributed to feeding by *Riptortus clavatus* on soybean seed.



Fig. 3. Symptoms of the CBD were revealed on the soybean sprout by subjecting the healthy hard soybean seeds to bean bug feeding prior to sprouting, soybean seedlot samples sprouted for 7 days by watering the container for 3 minutes so as to overflow for 30 seconds at every three hour at $22\pm 2^{\circ}\text{C}$.

lesion with white depressed center appeared on the cotyledon of soybean seed imbibed. The lesion did not develop further but turned darker in edges due to oxidation along with sprouting process. Coleoptile tissues were damaged by the sucking of bean bugs, so the lesion was sunken to depressed and produced cavity under seed coat, which is still intact.

The longer feeding periods, the higher CBD rate

One week feeding resulted in 41% incidence. The CBD did not concur with any other deleterious effects on sprouting such as hypocotyls elongation and rooting except unsightly sprout quality (Fig. 4, 5). The soybean pods were fed artificially at green pod, green-yellow pod, and yellow

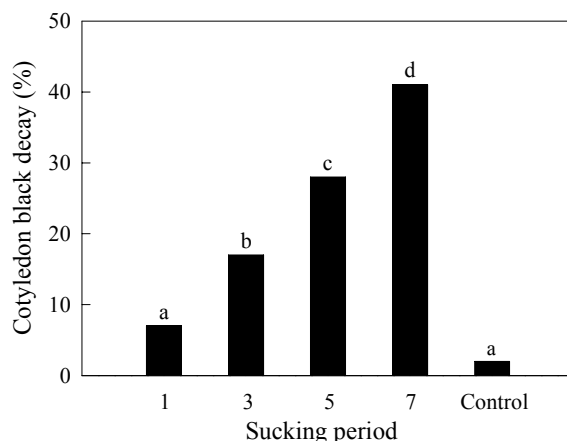


Fig. 4. Cotyledon black decay incidence of soybean sprouts in response to sucking period of hard healthy soybean seeds by bean bug. Bars corresponding to different letters differ significantly at $p = 0.05$.

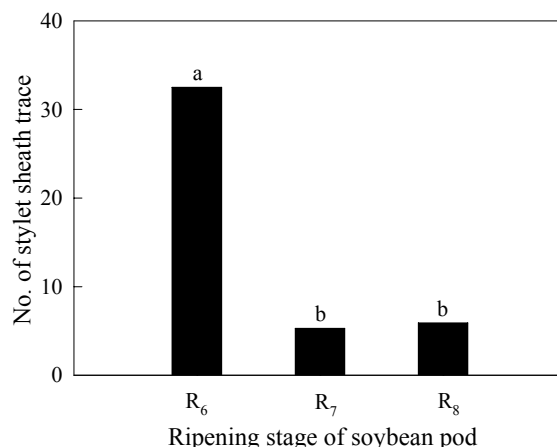


Fig. 5. Changes of stylet sheath trace on the 10 soybean seeds subjected to feeding soybean pods by bean bug at the different ripening stage. Bars corresponding to different letters differ significantly at $p = 0.05$.

pod stages, respectively: The trace of stylet sheath of bean bug was confirmed as average of 32.5 sites per ten seeds from green pod (R₆ stage), which was suggestive of repeated attack on a given site. The germination was limited to as low as 58%, which was the lowest of all and none of them revealed CBD (Data not shown). Those fed at R₇ and R₈ stage resulted in much less to an average of 5.3 and 5.9 stylet sheaths, respectively. However, germination rate was lower at 74% for R₇ stage compared to that of 83% at R₈ stage, which was attributed to the full maturity of seeds being more tolerant to bean bug feeding damage (Fig. 5 & 6).

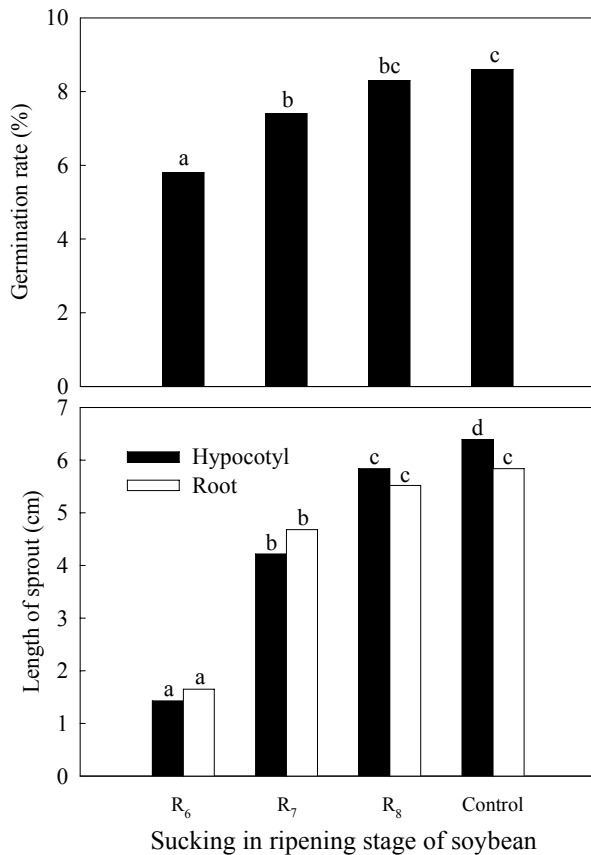


Fig. 6. Germination rate and growth status of soybean sprouts subjected to feeding soybean pods by bean bug at the different ripening stage. Bars corresponding to different letters differ significantly at $p = 0.05$.

DISCUSSION

CBD on soybean sprout looks like the black spot due to microbial infection. It is not uncommon and rendering unsightly sprouts which discourages consumers to purchase it. CBD is not visible or observable at seedlot stage, i.e., before sprouting and shows up exclusively on cotyledon upon sprouting. Over the years, many attempts to identify the biotic causal agent directly from the CBD samples have not been successful. The commercial field plots for soybean seedlot production were also monitored periodically for any clue that might be associated with CBD. Moreover, the symptom was not prevented by the heat treatment method developed by our team (Lee *et al.*, 2007a). Of the many commercial seedlot under storage, those seedlots samples produced in 2004 revealed the CBD at higher rates (Fig. 1). Therefore, the annual difference in incidence was attributed

to some variable factors associated with that specific year.

Since we confirmed CBD is carried over through seeds, we hypothesized some pod-infesting insect might have something to do with. Bean bugs suck the digested tissue material by the saliva pumped into the feeding plant tissue through the stylet (Miles, 1972). Accordingly, we paid attention to bean bug, *Riptortus clavatus*, one of the major pests sucking soybean pod. To prove this, the authors tried to feed the healthy hard soybean seed by bean bug in small cage in the laboratory (Fig. 2). Then seeds were grown to sprout in laboratory scale system, and obtained the CBD at the corresponding site of coleoptile where the bean bug fed previously, that are mimic to those occurred in soybean sprout from previous experiments on seedlots (Fig. 3). When the healthy hard seeds, fed by bean bug, were sprouted, only 41% of sprouts were recognized as being affected, i.e., CBD lesion confirmed. This result suggested the bugs preferred to suck the spot-site where the other bugs had fed previously, such that the given spot-site was attacked repeatedly. Hence, the frequencies of CBD lesion were lower than expected under experimental condition. This phenomenon was attributed to the aggregated feeding behavior of bean bug attracted to the aggregation pheromone. Numata *et al.* (1990) reported that both male and female bean bugs were more attracted to traps where adult males were maintained with green pods, compared to other traps with green pod or soybean seeds alone. They suggested some attractants involved in this phenomena, which were later elucidated as aggregation pheromone and being synthesized in laboratory (Leal *et al.*, 1995, Morishima *et al.*, 2005, Huh & Park, 2006).

Such a feeding behavior of bean bug might affect the size and shape of CBD lesion. The size of CBD lesion would depend upon frequency of bean bug attack at given site of seed. Thus the authors asserted that the CBD of soybean sprout was distinctively derived from seedlots with pod sucked by bean bug. Longer feeding promotes the rate of CBD on soybean sprout (Fig. 4). As Lee *et al.* (2004) reported, earlier feeding either at green pod or greenish yellow pod stage (R₆ - early R₇ stage) resulted in rather severe seed damages, which strikingly reduced hypocotyls growth to about one fourth to about two third, as well as the rates of seed germination. However, such an effect was not statistically significant in growth reduction in sprouts from full matured seeds

at late R₈ stages, suggesting matured seed was fairly tolerant to the sucking damages caused by bean bugs (Fig. 6) in terms of hypocotyl and root growth, but with still higher rate of 17% un-germination, compared to untreated control.

Much less incidence of average sucking damages at R₇ and R₈ stages, compared to that of R₆ stages suggested that pod yellowing is critical timing for seed maturing process, rendering more tolerant to bean bug feeding damage, in terms of germination rate and sprout growth (Fig. 5 & 6). Sucking at green pod stage resulted in more severe damage on germinating ability and sprout growth, which is attributed to the abnormality of cotyledon and embryo. The bean bug feeding damage on full matured healthy seed yielded distinct CBD on soybean sprout (Fig. 4). The mechanism involved in CBD development on cotyledon of soybean sprout well deserve further study.

Oh *et al.* (2007) reported that soybean sprout was remarkably of poor quality if sprouted from seedlot that has more than 5% damaged seeds. Many researches on the population dynamics and ecology of bean bug, were reported lately (Lee *et al.*, 2004; Bae *et al.*, 2005; Choi *et al.*, 2005; Huh *et al.*, 2005). Recently, Bae *et al.* (2005) attributed such a bean bug flourishes to the global weather changes. Quality control system should be worked out. None of them reported any results on CBD of soybean sprouts that is resulted from bean bug injured seeds. In this paper, we presented bean bug damage at ripening stage is directly responsible for the CBD syndrome of soybean sprout for the first time to the best of the authors knowledge to date.

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