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EFFECTS OF NEONICOTINOID AND METHOD OF BREAKING DORMANCY ON SEED GERMINATION AND SEEDLING VIGOUR OF OIL PALM (*Elaeis guineensis* Jacq.)

W CHANPRASERT*; T MYINT; S SRIKUL‡ and O WONGSRI‡**

ABSTRACT

The effects of two neonicotinoids at three application rates (0, 2.5 and 5.0 ml kg^{-1} seed for thiamethoxam, and 0, 2.0, 4.0 g kg^{-1} seed for imidacloprid) and two methods of breaking dormancy (dry heat treatment and operculum removal) on seed germination and seedling vigour were determined in tenera *oil palm seeds. The results reveal that the seeds with operculum removed took a shorter time to germinate than the dry-heated seeds, regardless of the neonicotinoid treatments. Thiamethoxam and imidacloprid had no promoting effects on seed germination or on time to 50% germination (T_{50}). In this study, phytotoxicity effects were observed with the neonicotinoid treatments in the operculum-removed seeds in terms of the percentage of seeds producing shoots and roots as compared to the normal seedlings (control); such effects were not found in the dry-heated seeds.*

Keywords: oil palm seed, germination, dormancy, thiamethoxam, imidacloprid.

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INTRODUCTION

Currently, oil palm is an important economic crop, supplying palm oil together with a variety of downstream production activities (Hooi *et al*., 2009; Poh *et al*., 2009), and making waves in the world market because of its suitability for use as biodiesel (Chandrasekaram *et al*., 2009) without contributing to an increase in carbon dioxide which causes global climate change (May *et al*., 2005). Growing global demand for palm oil has resulted in a tremendous increase in production area. For establishing new plantings, for replanting and the breeding programme, the availability of quality hybrid germinated seeds is essential (Martine *et al*., 2009). Palm seeds are a valuable resource for propagation (Orozco-Segovia *et al*., 2003), hence, the focus on oil palm seed germination still remains a priority for researchers.

Oil palm seeds have a dormancy period, leading to notably slow and a weak capacity for germination (Corley and Tinker, 2003). Rapid and uniform seed germination is a critical factor for oil palm plantations. A method to break dormancy would benefit commercial seed production and ensure the timely supply of planting materials (Murugesan *et al*., 2005). Despite dry heat having been accepted as a dormancy breaking method with intact seeds held at 40°C for 60 days (Addae-Kagyah, 1988), alternative treatments are still necessary to achieve rapid germination with low investment. From the little information that exists, the germination of oil palm seeds is mainly inhibited by the structure of operculum which is a mechanical impediment (Hussey, 1958; Nwankwo, 1981; Murugesan *et al*., 2008). In our previous report, a method of operculum removal was successfully developed to break dormancy in *tenera* hybrid seeds (Myint *et al*., 2010b).

* Department of Agronomy, Faculty of Agriculture, Kasetsart University, 10900 Bangkok, Thailand. E-mail: agrwcc@ku.ac.th
** Myanmar Perennial Crops Enterprise, Kabaraye Pagoda Road, 11061 Yangon, Myanmar.
‡ Surat Thani Oil Palm Research Center, Muaeng District, 84000 Surat Thani, Thailand.

As many factors may be manipulated for germination, there are various approaches to adopt with the target of obtaining faster seed germination in oil palm (Murugesan *et al*., 2005; Martine *et al*., 2009; Myint *et al*., 2009; 2010a). The response to growth regulators and chemicals has also been studied by Wan and Hor (1983) using gibberellic acid, and by Herrera *et al*. (1998) using hydrogen cyanamide and ethephon. In this study, neonicotinoid insecticides were applied as seed treatments as the effect of these insecticides in field crops has been shown due to their pronounced systemic action (Moser and Obrycki, 2009). The active compound of neonicotinoid is translocated and distributed throughout the growing plant upon application on the seed surface (Magalhaes *et al*., 2009). Besides the insecticidal effect of neonicotinoid (Nault *et al*., 2004; Ramesh and Ukey, 2006), published data on its effect on germination and growth are also available. Thiamethoxam and imidacloprid are insecticides classified in a broad-spectrum systemic neonicotinoid group (Moser and Obrycki, 2009) and their application as a seed treatment has been reported to increase seed germination and seedling vigour in some crops (Horii *et al*., 2007; Stevens *et al*., 2008). Therefore, the objectives of this study were to determine the effects of thiamethoxam (Cruiser®) and imidacloprid (Gaucho®) on seed germination and seedling vigour of oil palm seeds with dormancy broken by the dry heat treatment and the operculum removal method.

MATERIALS AND METHODS

Materials

The experiment was conducted in 2009 in the Surat Thani Oil Palm Research Centre (09°07'N, 99°21'E) located in southern Thailand. Seeds of *tenera* cross No. 37 [cross of Deli (C2120:184D×HC 133:1288D) and LaMe (IRH618:158T×HC129:1056P)], otherwise known as Surat Thani 2, were harvested and mechanically depericarped from the fruits. They were washed with detergent and submerged in a mixture of surfactant and sodium hypochlorite for 20 min, dipped into a mixture of benomyl and thiram, then left under shade for 24 hr. The seeds were divided into two groups for the two different methods of breaking dormancy (dry heat and removal of the operculum). Each group was further subdivided to accommodate the three rates each of two neonicotinoids, *i.e*. Cruiser® [thiamethoxim 35 FS {3-(2-chloro-1,3-thizol-5-yimethyl)-1,3,5-oxadiazinan-4-ylidene(nitro) amine}] and Guacho® [imidacloprid 70 WS {1-(6-chioro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine}]. The three rates were 0, 2.5 and 5.0 ml kg^{-1} seed for thiamethoxam and 0, 2.0, 4.0 g kg^{-1} seed for imidacloprid.

Neonicotinoid Seed Treatment with Dry-heated Seeds

As the moisture content of the seeds was determined to be about 18%, the air-dried seeds of each treatment were put in individual plastic bottles previously treated with ethyl alcohol, and then heated at 40°C for 60 days. After heating, seed moisture content was determined again.

Chemically treated seeds. According to the determined seed moisture content, the dry-heated seeds needed nine days of soaking in order to raise the moisture content to around 22% which is the optimum for seed germination. The seeds to be applied with chemicals were soaked in water for eight days, air-dried and weighed before being soaked in either of the two chemicals at the designated rates (thiamethoxam at 2.5 and 5.0 ml kg^{-1} seed and imidacolprid at 2.0 and 4.0 g kg^{-1} seed) for 24 hr. The treated seeds were then applied with a fungicide mixture (a mixture of benomyl 40 g and thiram 80 g in 5 litres of water), dried under shade for 2 hr, and then left to germinate in polyethylene bags measuring 37 cm × 26 cm kept in a germination room (ambient temperature).

Control seeds. To achieve the required nine days' soaking, the control seeds were soaked in water for eight days, air-dried, and weighed, and then soaked in water again for 24 hr. The soaked seeds were rinsed with water, washed with detergent, treated with the fungicide mixture, and surface dried under shade for 2 hr. The seeds were then germinated in polyethylene bags (similar in size to the above) kept in the germination room.

Neonicotinoid Seed Treatment with Operculum-removed Seeds

Chemically treated seeds. From the initial seed moisture content (without dry heat process) determined, the intact seeds required five days' soaking to raise their moisture content to about 22%. The seeds to be treated with the two different chemicals were soaked in water for four days, air-dried and weighed. After that the seeds were washed with detergent, submerged into a mixture of surfactant and sodium hypochlorite for 20 min, then spread under shade to dry for 2 hr.

The operculum of each seed was removed following the method developed by Myint *et al*. (2010b). The fibre plugs of the functional germ pores of the seed were removed by a knife sterilised with ethyl alcohol, then the germ pores were

widened by turning the blunt edge of the sterile knife around in the germ pore in the endocarp until a disc-like structure was reached. After that, the operculum (the disc-like structure together with the testa and a thin layer of endosperm) was removed by the sterile knife to expose the distal surface of the embryo.

After removing the operculum, the seeds were soaked in the respective chemical treatments (as detailed above) for 24 hr, then dipped in the fungicide mixture, dried under shade to remove superficial water around the seeds, and, finally, placed in the polyethylene bags in the germination room to germinate.

Control seeds. To satisfy the need to soak for five days, the intact seeds used as the control were soaked in water for four days, air-dried, weighed, and then resoaked in water for 24 hr. The seeds were washed with detergent and submerged in a mixture of surfactant and sodium hypochlorite for 20 min, then dried under shade for 2 hr.

After that, the operculum was removed from each seed, and the seeds were dipped in the fungicide mixture, dried under shade, then put in the polyethylene bags and germinated in the germination room.

Statistical Analysis

The experiment was performed in a 2 × 2 × 3 factorial using a completely randomised design (CRD) with four replications. Each replication consisted of 40 seeds for each experimental group of the dry heat treatment and 25 seeds for each of the operculum removal treatment. The two dormancy-breaking methods (operculum removal and commercial dry heat), the two neonicotinoid chemicals (thiamethoxam and imidacloprid) and their rates (two rates and zero control for each chemical) were designated as factors A, B and C, respectively. Moisture content was determined on five seeds per replication by oven-drying at 105°C for 48 hr (Panyangnoi *et al*., 1997) on a dry weight basis, before and after soaking the seeds in water. Seeds were checked for germination twice a week until six weeks after the seeds were placed in the germination room. Germination percentage (%G) was calculated according to the following formula (Martine *et al*., 2009):

$$\% G = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds tested}} \times 100$$

At the end of experimental period (six weeks in the germination room), the percentage of germinated seeds which had produced shoots and roots were counted, and shoot and root lengths were measured in mm. Time to 50% germination (T_{50}) was calculated according to the following formula of Coolbear *et al*. (1984):

$$T_{50} = t_i + \left[\left\{\frac{\frac{(n+1)}{2} - n_i}{n_j - n_i}\right\} \times (t_j - t_i)\right]$$

where t_i = time before reaching 50%;
n_i = number of seeds emerged at time t_i;
N = final number of emerged seeds;
t_j = time next to t_i; and
n_j = number of seeds emerged at t_j.

Cumulative percentage germination was recorded for each experimental unit. All data were subjected to an analysis of variance (ANOVA). The least significant difference (LSD) between means was calculated when the F test of ANOVA for treatments was significant at the 0.05 or 0.01 probability level.

RESULTS AND DISCUSSION

Effect of Neonicotinoid on Percentage of Germination and T_{50}

As shown in *Table 1*, thiamethoxam was compared with imidacloprid using the dry-heated seeds and the operculum-removed seeds. Throughout this experiment, germination was not significantly different due to either thiamethoxam or imidacloprid. Although both neonicotinoids gave almost the same result, a stimulative or inhibitive effect on germination was shown by the use of thiamethoxam and imidacloprid. Horii *et al.* (2007) and Cataneo *et al*. (2010) reported that in corn and soyabean, thiamethoxam accelerated germination. Decreased germination and increased occurrence of abnormal seedlings following seed treatment with imidacloprid were reported by Taylor *et al*. (2001) in onion and by Kuhar *et al*. (2002) in sweet corn. Positive or negative responses to other chemicals such as H_2SO_4, GA_3, ethephon and hydrogen cyanamide on germination have been detected in oil palm seeds (Corley, 1976; Wan and Hor, 1983; Herrera *et al*., 1998) and in Alexandra palm seeds (Nagao *et al*., 1980). From the present findings, the high germination might not have been the result of the use of neonicotinoids as there were no differences between the control and the two chemical rates. In this study, neonicotinoid chemicals were applied to the seed after their dormancy was removed. Perhaps such seeds would already possess physiological and metabolic functions for germination, and, thus, the promoting effect of neonicotinoid chemicals as a stimulus in germination was not significantly expressed.

Uniformity in terms of response to thiamethoxam and imidacloprid also occurred for T_{50}, which was

TABLE 1. EFFECT OF THIAMETHOXAM AND IMIDACLOPRID AT DIFFERENT RATES ON PERCENT GERMINATED SEEDS AND T_{50} (days) IN DRY-HEATED AND OPERCULUM-REMOVED SEEDS OF *Tenera* OIL PALM CROSS NO. 37 (Surat Thani 2)

Treatment	% germinated seeds			T_{50} (days)		
	DH	ROp	Average	DH	ROp	Average
Thiamethoxam (ml kg^{-1} seed)						
0.0	83.75	89.50	86.63	17.86	1.82	9.84
2.5	85.00	92.00	88.50	17.03	2.05	9.54
5.0	81.88	93.00	87.44	17.71	2.08	9.90
Imidacloprid (g kg^{-1} seed)						
0.0	80.63	93.00	86.81	17.95	2.08	10.02
2.0	80.63	87.00	83.81	17.46	2.06	9.76
4.0	81.25	92.00	86.63	19.45	2.21	10.83
Average	82.19 B	91.08 A	-	17.91 B	2.05 A	-
$LSD_{0.05}$ for						
Method (M)		4.30			0.57	
Chemical (C)		ns			ns	
Rate (R)		ns			ns	
M×C		ns			ns	
M×R		ns			ns	
C×R		ns			ns	
M×C×R		ns			ns	
CV%		8.5			13.4	

Note: means followed by different uppercase letters are significantly different from one another at the probability level of p = 0.05.
DH = dry heat method with heating at 40°C for 60 days; ROp = removal of operculum method.

9.76 and 10.20 days, respectively. The T_{50} of the untreated seeds as well as the seeds treated with the two concentrations of neonicotinoids was also very similar (*Table 1*).

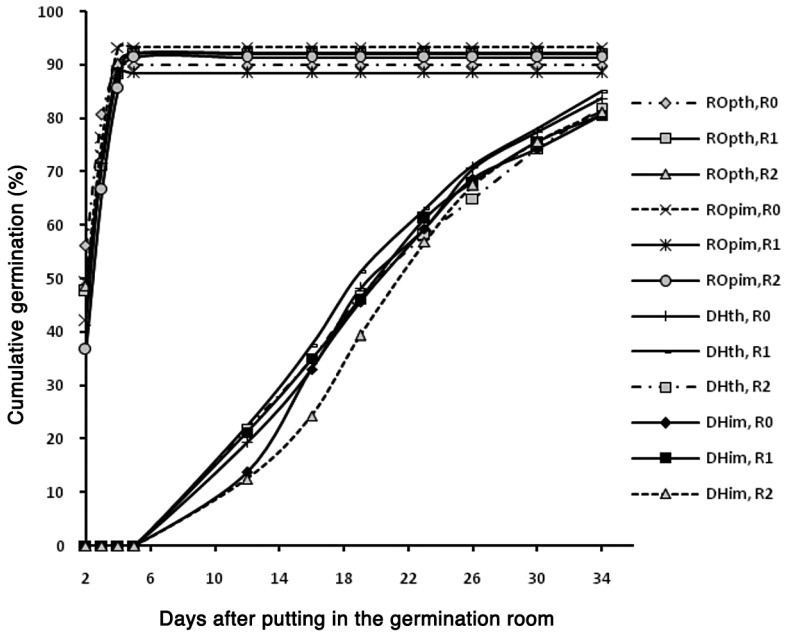
Both dormancy-breaking methods resulted in high germination. A distinct advantage in the operculum removal method was shown from the earlier and more synchronised germination to a large extent (*Table 1*). Operculum-removed seeds were capable of competing with commercial dry-heated seeds in the percentage of germinated seeds, at 91.08% compared with 82.19%, respectively. The speed of germination could also be accelerated by the removal of the operculum, as significant differences were clearly exhibited in T_{50}. The seeds from the dry heat treatment took 17.91 days whereas those with operculum removed germinated faster in 2.05 days (*Table 1*). This reduction of not only the difference in T_{50} of 15.86 days but also the heating period of 60 days was due to operculum removal. The result confirms the previous findings of Myint *et al.* (2010b) and supports the idea that operculum removal may be developed as an alternative method for breaking dormancy in commercial oil palm seeds. It may no longer be necessary to incubate the seeds in a heating room for 60 days as this method can obviously overcome the limitations of the dry heat method (*i.e*. costly, laborious and time-consuming). The operculum removal method only needs trained labour to remove the operculum without any damage to the embryo.

Cumulative Germination

As shown in *Figure 1*, the seeds with their operculum removed and soaked in neonicotinoids at the two rates together with the control seeds showed no variation in the first sign of germination, with all of them attaining approximately 45% germination by the second day. Germination was completed within five days, achieving around 90%. Application of neonicotinoids at the two rates to the seeds which were dry heat treated and the control seeds also did not produce any differences, but tended to start germinating in the second week and at a lower rate of about 20%, finishing after about five weeks with values of about 80% germination. More importantly, the time of initial germination and time to completion were significantly different between the seeds treated by the two dormancy-breaking methods.

Effects of Neonicotinoid on Germinated Seeds Producing Shoots and Roots

For the germinated seeds producing shoots and roots that were counted at the end of germination period (six weeks after putting them in the germination room), it was found that there were no significant differences between the controls of the dry heat treatment and the operculum removal method (83.75% *vs*. 79.50% for thiamethoxam and 80.63% *vs*. 78.00% for imidacloprid groups,



Note: ROpth R_0, R_1 and R_2 = operculum-removed seeds soaked in thiamethoxam at 0.0, 2.5 and 5.0 ml kg^{-1} seed, respectively.
ROpim R_0, R_1 and R_2 = operculum-removed seeds soaked in imidacloprid at 0.0, 2.0 and 4.0 g kg^{-1} seed, respectively.
DHth R_0, R_1 and R_2 = dry-heated seeds soaked in thiamethoxam at 0.0, 2.5 and 5.0 ml kg^{-1} seed, respectively.
DHim R_0, R_1 and R_2 = dry-heated seeds soaked in imidacloprid at 0.0, 2.0 and and 4.0 g kg^{-1} seed, respectively.

Figure 1. Cumulative germination percentage of the seeds from the operculum removal and dry heat methods soaked in thiamethoxam and imidacloprid at three rates.

respectively, producing shoots and roots), and also none between the two rates of both neonicotinoids in the dry-heated seeds (85.00% *vs.* 81.88% for thiamethoxam, and 80.63% *vs.* 81.25% for imidacloprid) (*Table 2*). However, the application of neonicotinoids caused an obvious deleterious effect on the shoots and roots of the germinated seeds of the operculum removal method resulting in only 32.00% and 33.00% for the two rates of thiamethoxam, and 38.00% and 28.00% for the two rates of imidacloprid (*Table 2*). This effect may be due to the phytotoxicity of the neonicotinoids on the seeds where the embryos were exposed by the operculum removal treatment. Serious phytotoxic effects of neonicotinoid on the growth and vigour of seedlings were also reported in *Carrot psylla* by Fischer and Terrettaz (2002). Phytotoxic responses to neonicotinoid seed treatment are affected not only by the active chemical being applied but also by factors such as formulation type, exposure period and conditions, crop species and cultivar (Stevens *et al*., 2008). However, Wilde *et al*. (2001) and Addison and Fisher (2002) reported that there were no phytotoxic or adverse effects on the growth of crops such as wheat and forage brassicas receiving neonicotinoid seed treatment; thus, the deleterious effect in this study may have resulted from imbibitional injury because of the treatment procedure involving soaking the operculum-removed seeds in neonicotinoid solution. Buchholz and Nauen (2002) reported that the application of neonicotinoid as a seed treatment can maximise its uptake into plant tissues. Our results could therefore be probably interpreted as a consequence of imbibitional stress to the exposed embryo that caused damage to the internal seed structures. Such imbibitional damage in other crops has been reported by Powell and Matthews (1980), Msanga and Maghembe (1989) and Pena-Valdivia *et al*. (2002). Rees (1960) reported that in oil palm seeds, excess moisture may also become an obstacle to germination because of improper ventilation (inadequate oxygen) for the physiological process of germination, or because moisture promotes the development of pathogenic fungi. From our observations, for the operculum-removed seeds which were soaked in water for 24 hr, some seeds died, with their embryos turning black while some remained in their embryonic axis of about 1 mm length and did not develop further. According to Prasanna *et al*. (2004), thiamethoxam shows no phytotoxic symptoms even at higher rates when applied to intact cotton seeds. In this study, no deleterious effect was observed in the intact dry-heated seeds treated with thiamethoxam and imidacloprid. In these seeds with intact operculum, the uptake of thiamethoxam and imidacloprid might have been lower, or the embryos might not have been in direct contact with the chemicals when the seeds were soaked in neonicotinoid solution. Therefore, no damage to the shoots and roots was found, and it was evident that there was

TABLE 2. EFFECT OF THIAMETHOXAM AND IMIDACLOPRID AT DIFFERENT RATES ON PERCENT GERMINATED SEEDS PRODUCING SHOOTS AND ROOTS, SHOOT LENGTH (mm) AND ROOT LENGTH (mm) AT SIX WEEKS AFTER PLACING DRY-HEATED AND OPERCULUM-REMOVED SEEDS OF *Tenera* OIL PALM CROSS NO. 37 (Surat Thani 2) IN THE GERMINATION ROOM

Treatment	% germinated seed producing shoots and roots			Shoot length (mm)			Root length (mm)		
	DH	ROp	Average	DH	ROp	Average	DH	ROp	Average
Thiamethoxam (ml kg^{-1} seed)									
0.0	83.75	79.50	81.63 a	5.28	6.07	5.67	20.23	20.18	20.20 a
2.5	85.00	32.00	58.50 b	5.63	5.91	5.77	21.20	16.32	18.76 a
5.0	81.88	33.00	57.44 b	6.23	6.15	6.19	22.18	16.89	19.53 a
Imidacloprid (g kg^{-1} seed)									
0.0	80.63	78.00	79.31 a	5.38	6.13	5.75	18.18	19.42	18.80 a
2.0	80.63	38.00	59.31 b	6.58	5.25	5.91	19.48	22.93	21.20 a
4.0	81.25	28.00	54.63 b	4.58	6.80	5.69	15.13	14.55	14.84 b
Average	82.19 A	48.08 B	-	5.61 B	6.05 A	-	19.40 A	18.38 A	-
$LSD_{0.05}$ for									
Method (M)		5.13			0.43			ns	
Chem (C)		ns			ns			ns	
Rate (R)		6.28			ns			2.05	
M×C		ns			ns			2.37	
M×R		8.88			0.75			ns	
C×R		ns			ns			2.9	
M×C×R		ns			1.06			ns	
CV%		9.8			12.7			15.1	

Note: means followed by the same upper case letter within a row, and lower case within a column, do not differ significantly from each other at the probability level of p = 0.05.
DH = dry heat method with heating at 40°C for 60 days; ROp = removal of operculum method.

no abnormality in root and shoot development following neonicotinoid treatments in the dry-heated seeds.

As shown in *Table 2*, there were no variations in shoot length when the seeds were submerged in thiamethoxam or imidacloprid. No significant difference was found in shoot length among three rates of each neonicotinoid. The significant difference in shoot length was found between the two methods of dormancy breaking, at 5.61 and 6.05 mm for the dry-heated seeds and operculum-removed seeds, respectively. No significant difference between dormancy-breaking methods was exhibited in the case of root length. There was also no significant difference among the three rates of thiamethoxam, but there was a significant difference among the seeds treated with imidacloprid, with the shortest root length recorded at the higher concentration of 4 g kg^{-1} seed. The response to thiamethoxam and imidacloprid was reported to be positive in some crops but negative in others (Kuhar *et al*., 2002; Ester *et al*., 2003; Horii *et al*., 2007). Horii *et al*. (2007) suggested that neonicotinoids may cause positive/negative effects on seed vigour depending on the concentration of the treatments. Therefore, lower concentration, shorter soaking time or different treatment method with neonicotinoids should be taken into considerations in future experiments aiming to improve seed germination performance and seedling vigour of oil palm.

CONCLUSION

Neonicotinoids such as thiamethoxam and imidacloprid did not significantly promote germination and T_{50} of *tenera* oil palm seeds. Operculum-removed seeds performed better in terms of germination, and showed a faster T_{50} over the dry-heated seeds, regardless of the effect of the neonicotinoids. Phytotoxicity was observed only in the operculum-removed seeds which received either thiamethoxam or imidacloprid treatments, showing lower percentages of germinated seeds producing shoots and roots. The deleterious effect by the neonicotinoids was not found in the dry-heated seeds.

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