PREDOMINANCE OF BLOW FLIES (DIPTERA: CALLIPHORIDAE) AMONG INSECTS VISITING FLOWERS OF *BUCHANANIA LANZAN* (SAPINDALES: ANACARDIACEAE)

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Abstract. This study highlighted blow flies as new possible pollinators of *Buchanania lanzan* (Sapindales: Anacardiaceae) flowers. Two sets of 2-day sampling were done by using a sweep net on the flowers of *B. lanzan* in a deciduous forested area of Mahidol University, Nakhonsawan Campus, Nakhon Sawan Province, Thailand during 27-28 December 2013 and 13-14 December 2014. The number of flies (Diptera) collected was larger than the combined number of bees (Hymenoptera) and beetles (Coleoptera) by a factor of 9.4. Blow flies (Diptera: Calliphoridae) were the most abundant insects caught (34.8% of total), and included the medically-important *Chrysomya megacephala*. *Stomorhina discolor* predominated (23.6% of total), followed by *C. megacephala*, *C. rufifacies*, *C. nigripes* and *Rhyncomya flavibasis*. Female flies predominated over males and were mostly collected in the morning. Many pollen grains were attached to the bodies of the flies, especially on the legs, thorax, and/or abdomen, indicating the likely importance of blow flies as pollinators of the blossoms of *B. lanzan.* These new findings could be useful for furthering conservation management and economic production of *B. lanzan,* and also for the use of *B. lanzan* flowers or the chemicals which they extrude as bait for controlling medically-important fly populations.
Keywords: *Almondette, insect diversity, pollinator, Stomorhina discolor, wasp-mimicking flies*

Introduction

Buchanania lanzan (Spreng) (common name Almondette, family Anacardiaceae) is found growing naturally as a wild plant in subtropical and tropical deciduous forests in many countries, including Thailand (Wongpakam et al., 2007; Sereesongsaeng and Khumchompoo, 2009), Myanmar, Malaysia, India (Martin et al., 1988), Australia and the Pacific islands (Chauhan et al., 2012). The tree is moderate-sized with alternate and simple leaves. Many parts of *B. lanzan* have socio-economic or medicinal importance, particularly in India. Its kernel is a commercially edible nut and is regarded as substitute for almonds in desert habitats (Rosengarten, 1984). Prasad and Bhatnagar (1993) reported that more than one thousand tons of kernels were harvested yearly from native forests of Mahdya Pradesh and Chhattisgarh in India (Duke, 2000). The other parts of this plant are of phytomedical and pharmaceutical importance and are used in Indian folk medicine to treat wounds, skin diseases, and diarrhoea (Patsnaik et al., 2011; Kumar et al., 2012). The bark may also be beneficial for application under conditions of immune stimulation and/or bacterial infection (Sekhar et al., 2015). Unfortunately, no commercial farming of *B. lanzan* has been carried out. Thus, ecological management may be needed to balance the demands of medicinal utility and tree conservation (Chauhan et al., 2012).

During reproduction, the flowers are formed in a panicle. As in the mango, *Mangifera indica*, both staminate flowers and hermaphrodite flowers are present in each panicle, but pollination is achieved by neither self-propagation nor wind pollination.

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Insect pollination is therefore presumed to play a significant role in propagation (Huda et al., 2015).

Blow flies (Diptera: Calliphoridae) are considered to be of medical and veterinary concern, and to have negative impacts on public health worldwide (Greenberg, 1973). From an ecological and agricultural standpoint, however, blow flies are potentially important pollinators of flowers including mango, from which they gather pollen or nectar on their legs and body surfaces during feeding (Anderson et al., 1982; Hu et al., 1995).

At Mahidol University Nakhonsawan Campus, when *B. lanzan* trees were blooming during December 2012, casual visitors to the vicinity of the trees assumed that the buzzing sound was from bees or wasps. After conducting preliminary observations, however, first author found that almost all of insects at the tree were flies such as *Chrysomya megacephala*, *Chrysomya rufifacies* and *Stomorhina discolor*. The objective of this study was therefore to determine the relationship between blow flies and flowers of *B. lanzan.*

Materials and Methods

Study site

Insect sampling was conducted at a *B. lanzan* tree on the Mahidol University Nakhonsawan Campus, Nakhon Sawan Province, north-central Thailand for a two-day period during two successive dry seasons (27–28 December 2013 and 13–14 December 2014), coinciding with the flowering period of *B. lanzan*. The *B. lanzan* tree sampled was located outside the administrative offices of the university (15.57912°N, 100.43717°E) (*Figure 1A*).

Shorea obtusa (Wall. ex Blume.) and *Shorea siamensis* (Miq.) have been found commonly near the study site. They are common trees in a disturbed deciduous dipterocarp forest. In a radius of 20 m, an additional 10 trees of *Azadirachta indica* (A. Juss.) (3), *Diospyros rhodocalyx* (Kurz.) (1), *Maerua siamensis* (Kurz) Pax. (3), *Pterocarpus indicus* (Willd.) (2) and *S. obtusa* (1) were found.

Weather data collected at Nakhonsawan Meteorological Station (15.67213°N, 100.12962°E), ~10 km distance from the study site, showed that daytime maximum temperatures reached 33.8 °C and 35.5 °C during December 2013 and 2014, respectively (www.met-sawan.tmd.go.th). Rainfall was c. 2.4 mm during December 2014, while; no rain fell during December 2013.

Insect sampling on B. lanzan flowers

A sweep net was used to catch all insects swarming over the flowers of *B. lanzan* during 3 h in the morning (0900-1200 h) and 3 h in the afternoon (1300-1600 h), in order to examine the pattern of insect visitation. All insects collected were anesthetized in an ethyl acetate killing jar. The insects were sorted to family using the taxonomic key of Triplehorn and Johnson (2005), and then identified to genus and species (where possible) under a stereo microscope (Olympus, Japan). Blow flies and muscid flies were identified using the taxonomic keys of Kurahashi and Bunchu (2011) and Tumrasvin and Shinonaga (1977), respectively. Unknown species were grouped as morpho-species.

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All insects were pinned and observed for attachment of pollen under the stereo microscope. Finally, all insects were deposited in preservation boxes at Mahidol University Nakhonsawan Campus.

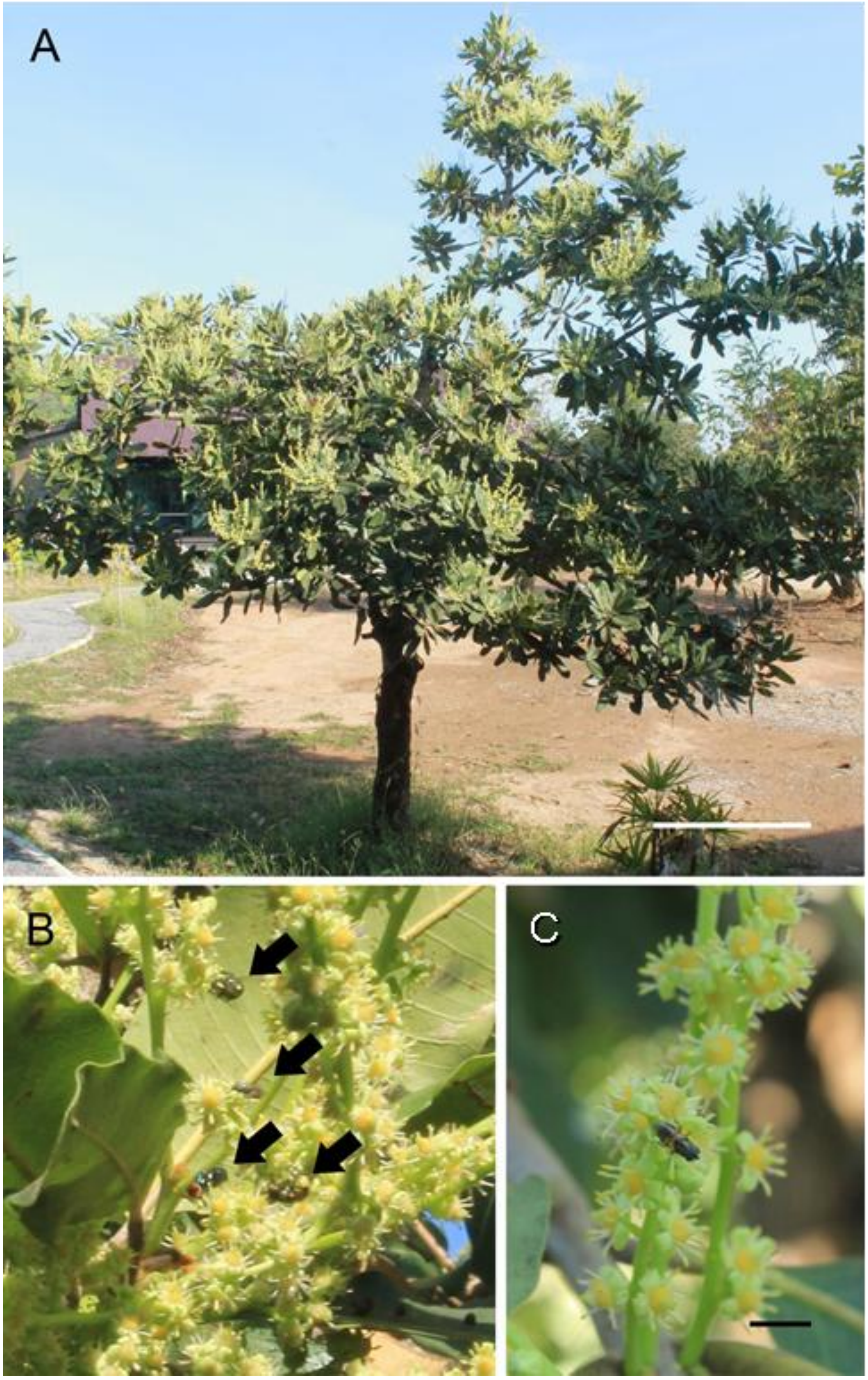

***Figure 1.** The sampled Buchanania lanzan tree, located near the administrative offices of Mahidol University Nakhonsawan Campus, Nakhonsawan Province, Thailand; (A) the tree with flowers (bar = 1 m); (B) various blow fly species visiting the blossom (black arrow heads); and (C) Stomorhina discolor, the predominant species visiting blossoms (bar = 1 cm)*

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Data analyses

The numbers of individuals of each insect species collected from the *B. lanzan* tree were counted.

Statistical analysis was performed using SPSS 17.0 for Windows. ANOVA was used to determine the difference among numbers of flies (males vs. females) collected during morning (0900-1200 h) and afternoon (1300-1600 h) ($p < 0.05$).

Results

Diversity of insects on B. lanzan flowers

A total of 843 insects were collected during investigations (*Table 1*). The numbers of insects collected between years were quite similar, with 404 insects in 2013 and 439 insects in 2014. Insects were sorted into 3 orders. Flies (Diptera) ranked as by far the most abundant insects, while the combined number of bees (Hymenoptera: *Apis mellifera*) and beetles (Coleoptera) contributed only 3.7% of the total.

Table 1. *Classification and total number (%) of insect species visiting flower of Buchanania lanzan at Mahidol University Nakhonsawan Campus, during 2-day sampling of 27–28 December 2013 and 13–14 December 2014*

Order	**Family**	**Species**	**No. (%)**
Diptera	Calliphoridae	*Stomorhina discolor* (Fabricius, 1794)	199 (23.6)
		Chrysomya megacephala (Fabricius, 1794)	57 (6.8)
		Chrysomya rufifacies (Macquart, 1843)	30 (3.6)
		Ceynolomyia nigripes (Aubertin, 1923)	5 (0.6)
		Rhyncomya flavibasis (Senior-White, 1922)	2 (0.2)
	Syrphidae	Undetermined	156 (18.5)
	Stratiomyidae	*Hedriodiscus* sp.	116 (13.8)
	Drosophilidae	*Drosophila melanogaster* Meigen, 1830	96 (11.4)
	Muscidae	*Graphomya rufitibia* Stein, 1918	66 (7.8)
		Musca sorbens Wiedeman, 1830	19 (2.2)
	Phoridae	*Megaselia scalaris* Loew, 1866	12 (1.4)
	Sarcophagidae	*Boetcherisca peregrina* Robineuau-Desvoidy, 1930	4 (0.5)
		Parasarcophaga dux Thomson, 2867	3 (0.4)
		Sarcophaga spp. Meigen, 1826	2 (0.2)
	Tachinidae	Tachinid fly	7 (0.8)
	Tabanidae	*Tabanus* sp. Linnaeus, 1758	3 (0.4)
	Undetermined	Undetermined Diptera	35 (4.2)
Hymenoptera	Apidae	*Apis mellifera* Linnaeus, 1758	24 (2.8)
Coleoptera	Undetermined	Undetermined	7 (0.8)
Total			**843 (100)**

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Ten families of flies were found on *B. lanzan* flowers (*Table 1*). Of these, blow flies (Calliphoridae) were the most abundant (34.8% of total) and species-rich (R = 5 species). These comprised *Stomorhina discolor* (*Fig. 1B-C*), *Chrysomya megacephala* (*Fig. 1B*), *Chrysomya rufifacies*, *Chrysomya nigripes*, and *Rhyncomya flavibasis*.

Syrphid flies (Syrphidae) were the second most abundant (18.5% of total; R = 4) and comprised 4 morpho-species. *Hedriodiscus* sp. (Stratiomyidae), *Drosophila melanogaster* (Drosophilidae), and *Graphomya rufitibia* and *Musca sorbens* (Muscidae) all contributed high numbers in our samples. Otherwise, *Megaselia scalaris* (Phoridae), *Boetcherisca peregrina*, *Parasarcophaga dux*, *Sarcophaga* sp. (Sarcophagidae), and other undetermined Diptera were much less abundant. Blood-sucking flies (Tabanidae: *Tabanus* sp.) contributed just 3 individuals (*Table 1*).

Visitation of insects on B. lanzan flowers

The number of insects visiting the flowers of *B. lanzan* was higher during the morning (0900-1200 h) than the afternoon (1300-1500 h) (*Fig. 2*). The difference in the numbers visiting between morning and afternoon periods was significant in the blow flies *S. discolor* and *C. megacephala* (p = 0.002 and 0.023, respectively). Significant differences were also found in *Hedriodiscus* sp., *D. melanogaster*, *G. rufitibia*, and syrphid flies ($p < 0.05$). No significant difference was found in the diurnal visitation pattern of *A. mellifera* or beetles, while *R. flavibasis* and *Tabanus* sp. were only found during the morning period.

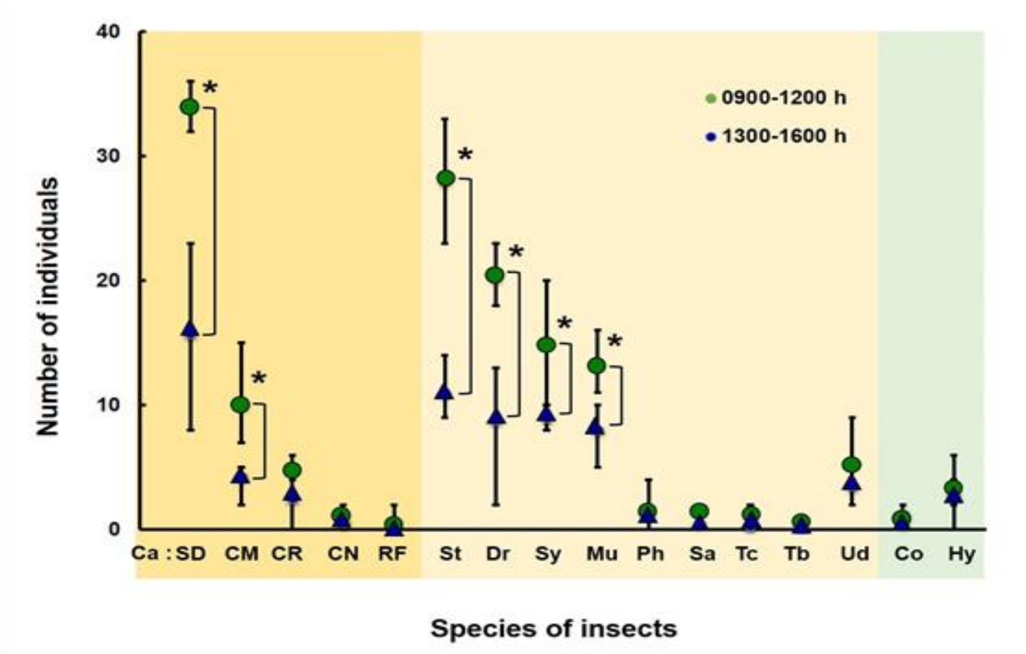

Figure 2. *Numbers and visitation times of insects sampled on flowers of Buchanania lanzan at Mahidol University Nakhonsawan Campus, during sampling, 27–28 December 2013 and 13–14 December 2014 (n = 4) (Ca Calliphoridae, SD Stomorhina discolor, CM Chrysomya megacephala, CR Chrysomya rufifacies, CN Chrysomya nigripes, RF Rhyncomya flavibasis, St Stratiomyidae, Dr Drosophilidae, Sy Syrphilidae, Mu Muscidae, Ph Phoridae, Sa Sarcophagidae, Tc Tachinidae, Tb Tabanidae, Ud undetermined Diptera, Co Coleooptera, Hy Hymenoptera); *indicates significant differences in numbers of insects visiting between 0900–1200 h and 1300–1600 h (independent T–test, p < 0.05)*

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More female flies were caught than males, although this difference was not significant ($p > 0.05$) in most. In muscid flies and sarcophagid flies males predominated over females ($p > 0.05$).

Occurrence of pollens on insect bodies

Forty-five percent of insects sampled had pollen on their bodies (*Table 2*). The exceptions were the tiny flies of *D. melanogaster*, *M. scalaris*, and *C. nigripes*, blood-sucking *Tabanus* sp., and beetles.

Table 2. *Percentage of insects with pollen on their bodies at Mahidol University Nakhonsawan Campus during periods of 27–28 December 2013 and 13–14 December 2014*

Order	**Family or Species**	**% of insects with pollens**	**% of insects bear organs with pollens**				
			Proboscis	**Eyes**	**Thorax**	**Legs**	**Abdomen**
Hymenoptera	*Apis mellifera*	75.0	0	0	50.0	37.5	33.3
Diptera	Tachinidae	71.4	14.3	0	57.1	57.1	42.9
	Graphomya rufitibia	59.1	10.6	13.6	34.8	30.3	18.2
	Stomorhina discolor	58.3	6.5	0	48.7	11.1	13.1
	Hedriodiscus sp.	54.3	0	0	31.9	24.1	15.5
	Syrphidae	52.6	3.2	5.1	30.8	23.1	26.3
	Rhyncomya flavibasis	50.0	50.0	0	50.0	0	0
	Chrysomya megacephala	43.9	14.0	0	12.3	33.3	24.6
	Musca sorbens	42.1	0	0	26.3	21.1	42.1
	Chrysomya rufifacies	40.0	23.3	0	13.3	6.7	26.7
	Sarcophagidae	33.3	0	0	11.1	33.3	11.1
	Undetermined Diptera	20.0	0	0	11.4	14.3	0
	Total	**45.0**	**5.0**	**2.0**	**28.8**	**18.0**	**16.5**

The small number of honey bees *A. mellifera* (24 bees) ranked first among the percentage of individuals bearing pollen (75%), found on thorax, legs and abdomen. They were followed by tachinid flies (71.4%) and *G. rufitibia* (59.1%), consecutively. *S. discolor*, the most abundant fly sampled in this study, ranked fourth (58.3% bore pollen). In flies of these three families pollen was often present on the proboscis in addition to thorax, legs and abdomens. Additionally, pollen grains were attached to the minute hairs located between the facets of compound eyes of both muscid flies, *G. rufitibia*, and syrphid flies.

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Discussion

The two years' observations revealed that *S. discolor* was the most common blow fly found around opened flowers of *B. lanzan*. The relationship between this fly species and the flowers of *B. lanzan* has not been previously documented. The length of the pollinator's proboscis is generally related to shape of the flowers they visit (Tangmitcharoen and Owens, 1997). A short proboscis, as found in flies and bees, is usually associated with feeding on already opened flowers (Larson et al., 2001). In addition, different insect pollinators are attracted to flowers of different colors and shades. Flowers with dull colors, such as those of *B. lanzan*, tend to attract fly pollination; while, bright colors mostly attract bees (Shivanna and Tandon, 2014).

In this study, flies preferred to visit flowers in the morning rather than in the afternoon, recalling the findings of Brown and McNeil (2009) for cloudberry *Pubus chamaemorus*. The peak of diurnal activity differs among different insect species and is usually correlated with the diurnal blooming peak of particular plant hosts. Peak blooming of mango, for example, tends to occur during 0900-1100 h (Huda et al., 2015). The timing of the blooming of *B. lanzan* in relation to *S. discolor* visitation requires further investigation.

Although pollen was found attached to the fly specimens collected in this study, whether any of these fly species is an actual pollinator of this tree is still in question. Banziger et al. (2008) found that despite the fact that blow flies (*Calliphora vomitoria* (Linnaeus) and *Calliphora pattoni* Aubertin) entered the lip of the lady slipper orchids *Cypridedium yunnanense* and *C. flavum* (Orchidaceae), they proved not to be pollinators, even when smeared with pollen. Nonetheless, there are other reports of blow flies as pollinators. For example, females of *Lucilia porphyrina*, *Chrysomya pinguis, Chrysomya chani* and *Chrysomya villeneuvei* were the main pollinators of *Rhizanthes zippelii* (Rafflesiaceae) in Thailand (Banziger, 1996). Howlett (2012) found that the European blue blow fly *Calliphora vicina* Robineau-Desvoidy was an effective pollinator of the hybrid carrot (*Daucus carota* L.) seed crop in New Zealand. In China, several species of blow flies (*C. megacephala*, *Isomyia isomyia*, *Pierretia* sp., *Hemipyrellia* sp. and *C. rufifacies*) were the most frequent visitors to flowers of *Bridelia stipularis* and *Cleistanthus sumatranus* on tropical Hainan Island (Li et al., 2014). Likewise, females of *Lucilia* and *Chrysomya* were major pollinators of *Rafflezia pricei* in northern Borneo of Malaysia (Beaman et al., 1988). Moreover, *C. megacephala* blow flies have been reared and used as pollinators of mango in Australia (Anderson et al., 1982) and Taiwan (Hu et al., 1995). In Hong Kong, China, Lau et al. (2009) revealed that *C. megacephala* provided more effective pollination of *Bauhinia* sp. (Leguminosae) than honey bees due to its more frequent rate of visitation. Greenhouse experiments would help verify whether *S. discolor* is truly a pollinator of *B. lanzan*.

In Thailand, the *B. lanzan* tree is little known although it is found in many parts, especially in the northeastern and central regions (Wongpakam et al., 2007; Sereesongsaeng and Khumchompoo, 2009). It is utilised by local people near Mahidol University Nakhonsawan Campus, who take the young leaves, young shoot tips and flowers of *B. lanzan*, eating them as a fresh vegetable with Thai dipping paste (chili paste).

Further, although the odor of *B. lanzan* flowers was not strong, unlike carrion flowers; it was remarkable that flies, including medically important blow flies such as *C. megacephala* and *C. rufifacies*, were attracted to visit. Therefore the odor-causing chemicals of *B. lanzan* flowers should be studied for possible application as a bait to trap those fly species when necessary for pest control. In conclusion, this study sheds light on the relationship of *B. lanzan* flowers and the blow fly *S. discolor* under natural conditions in Thailand. These new findings might be useful for further conservation management and economic production of *B. lanzan,* and also for the use of *B. lanzan* flowers such as the chemicals which they extrude as bait for controlling medically-important fly populations. Such information will help widen our knowledge of the roles of indigenous flies for improving agricultural practice in the future.

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